Th rap utic compositions comprising butyrospermol

Patent Number: GB932662
Publication date: 1963-07-31
Inventor(s): BOITEAU PIERRE; RATSIMAMANGA ALBERT RAKOTO
Applicant(s):: LAROCHE NAVARRON LAB
Requested Patent: ☐ GB932662
Application Number: GB19600013221 19600413
Priority Number(s): GB19600013221 19600413
IPC Classification:
EC Classification: A61K35/78
Equivalents:

Abstract

The substance "butyrospermol", i.e. 3b -hydroxy-(13a , 14b , 17b H)-lanosta-7,24-diene, is extracted from "shea-butter" (the kernels of the fruit of the W. African tree butyrospermum Parkii) with CCl4. The fatty materials obtained by evaporation of the solvent are taken up by sodium hydroxide dissolved in pure methanol and the methanol is removed to give a soap which is put on a chromatographic column which is extracted with CHCl3. Evaporation of the solvent gives a mixture of the triterpene alcohols butyrospermol, b -amyrin and "Parkeol" (3b -hydroxy-lanosta-9(11), 24-diene) which is acylated by acetic anhydride in the presence of pyridine and the acetates separated by fractional crystallization or by chromatography on alumina. Pure butyrospermol may then be obtained by hydrolysis of its acetate. ALSO: Pharmaceutical compositions for oral, parenteral and local application, e.g. as tablets, parenteral solutions, balms and ointments and having hormonal, cicatrizing and bactericidal activity comprise a carrier and the compound butyrospermol having the structure The butyrospermol may be extracted from the kernels of the fruit of butyrospermam Parkii (shea butter) or the oil mill cakes thereof or from the latex of the breadfruit tree. The extraction from shea-butter is described wherein the raw material is ground with CCl4, and the solvent is removed from the extracted fatty materials which are taken up in NaOH/CH3OH. The methanol is removed and the resultant soap is put in a chromatographic column which is extracted with CHCl3. By evaporation of the solvent a mixture is obtained comprising butyrospermol, b -amyrin and "Parkiol" which may be used in the formulation of the compositions or the butyrospermol extracted therefrom (see Group IV (b)).

COMPLETE SPECIFICATION
Therapeutic Compositions comprising Butyrospermol
We, LABORATOIRES LAROCHE a French Body Corporate of 63, Rue Chaptai,
Levallois (Seine), France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-
The present invention has for its object a new therapeutic composition endowed with hormonal properties, and moreover cicatrizing and bacteriddal properties.

According to this invention it has been discovered that butyrospermol, until now without practical application, possesses these various properties to a marked extent, making it an interesting therapeutic agent.

Butyrospermol, or 35-hydroxy-(13cc, 14,ss, 175 H)-lanosta-7,24 diene is a tetracyclic tri-terpene alcohol of formula C30H50O (MW 426.7) having the structural formula illustrated in the drawing accompanying the Provisional Specification.

It is extracted from the kernals of the fruit of butyrospermum Parkii a large tree common in West Africa, especially in the Sudan area, and known under the name of karite (sheabutter). It may equally well be extracted from the oil mill cakes of karite, in which it remains for the major part, or separated from the latex of the breadfruit tree, Artocarpus integrifolia (Jack fruit).

The raw material (shea-butter) after grinding is extracted with carbon tetrachloride. After evaporating the solvent, the whole of the collected fatty materials is taken up by sodium hydroxide dissolved in pure methyl alcohol.

The fatty adds, especially the palmitic acid, are thus converted into a soap insoluble in chloroform. After the methyl alcohol has been driven off, the resultant soap is put in a chromatographic column and extracted with chloroform until the solvent no longer gives a coloration by addition of Noller's reagent (antimony trichloride in thionyl chloride solution). By evaporating the resultant chloroform solution, a mixture of triterpene alcohols is obtained: butyrospermol, -amyrin and "Parkeol", of which mixture butyrospermol is the main constituent. Parkeol is a triterpene alcohol of formula C30H0O, the systematic nane of which is 3p-hydroxy lanosta-9 (II), 24 diene.

This mixture may thus be used as it stands for pharmaceutical purposes. However, if pure butyrospermol is desired, this mixture may beacetylated by acetic anhydride in the presence of pyridine. The acetates are then collected and may be separated either by virtue of their differences in solubility and hence by fractional crystallization, or by a fresh chromatography run on alumina. Upon separating the acetates, pure butyrospermol may be obtained by hydrolysis of its acetate.

Butyrospermol has the following characteristics:
m.p. = 108--113"C.

[a]n = 12.5 in chloroform solution.

It gives a yellowish-brown coloration with tetranitro-methane, a reddish-brown coloration accompanied by a characteristic green fluorescence with Liebermann-Bouchard reagent, and a pink coloration changing to purple with Noller reagent.

Butyrospermol gives inter alia the following derivatives: -Acetate: m.p. = 146--148"C.

[ajD = +11.5 :t2.5 in chloro
form solution -Benzoate:m.p. = 130-133 0C.

[OC]D = t33 .5 in chloroform solu-
tion - Cinnamate and palmitate, the latter having the advantage of being readily soluble in fatty materials: -
Butyrospermone or 3-oxo-(13c(, 14 , 17 H)-lanosta-7,24-diene
m.p.= 77-84 0C.

[olD = -40 #4 in chloroform

solution.

The pharmacological properties of butyrospermol will now be discussed. It has a threefold activity: it has harmonal, cicatrizing and bactericidal properties.

I -- HORMONAL ACTIVITY:
This is mainly a cortico-suprarenal activity.

It is related to the action of desoxycorticosterone (DOC-like-action) and to the action of cortisone (cortisone-like-action).

1 -- DOC-like action:
This action, in contrast with the action of desoxy-corticosterone is also effecdve on oral administration; it is evidenced by means of the following test. The survival without aggression is determined for male rats weighing 40 g and having undergone suprarenalectomy.

The animals are divided into two groups of 30, maintained at 30"C, and receiving at will balanced feed and, as drink, an aqueous solution of NaCl at 9% 0. However, this beverage is discontinued after 21 days of test. The.

first group is used to control, and the second group is administered butyrospermol, by gastric tube, twice daily. Table I shows the percentage of survivors in each group, with respect to time (in days) following suprarenalectomy.

TABLE I

Butyrospermol, 250 γ daily
Days of test Controls % by gastric tube - %
7th
8th
9th 100
10th 87 100
tilth 87 86
12th 87 71
13th 87 71
14th 50 71
15th 12 71
16th 12 71
17th 12 71
18th 12 71
19th 12 71
20th 12 71
21st 12 71
Suppression c f of salt water
22nd 12 71
23rd 12 71
24th 12 . 57
25th 12 43
26th 0 28
27th 28
28th 28
29th 28
30th 28
31st 0

2. - Cortisone-like action:
This action is evidenced by the survival test of male rats weighing 35 g, subjected to cold (+3 C.) 48 hours after having undergone suprarenalectomy.

The rats are divided into three groups of 20.

Two of the groups are administered, at regular 90 minute intervals, sub-cutaneous injections of 1 ml of water containing 10% alcohol and 5y and 500y, respectively, of butyrospermol.

The third group is used as control and is oniy administered the vehicle, at the same time intervals.

The Table II given farther shows, for each case, the percentage of surviving animals with respect to time following the beginning of the experiment.

TABLE II

Butyrospermol
Controls
5 5 Y 500 y
4 h 100
15 80
30 60
45 60 100
5 h 60 83
15 60 83
30 60 83
45 60 83
6 h 60 100 83
15 60 80 83
30 60 80 83
45 40 80 83
7 h 40 80 50
15 40 60 50
30 40 40 33 . .

45 40 0 33
8h 40 16
15 20 16
30 0 0
45
-Mean time of survival 6 h 27 7 h 16 7 h 10
% of increase over controls +12.27% +11.10%

II - CICATRIZING PROPERTIES:
1. Cicatrization test on the cornea of rabbit.

Similar trauma of the cornea, by means of a red-hot iron, are carried out on rabbits of same origin, of same weight, and having previously been submitted to local anaesthesia.

A first group of rabbits is used as control.

A second group of rabbits is given intramuscular injections of butyrospermol, which is administered twice weekly at a dosage of 30 mg. After 10 days, cicatrization is complete only in the rabbits which have been treated, and the cornea recovers its transparency.

2. Cicatrization test on experimental wounds in mice.

The rate of cicatrization and the death rate of male mice (25 g) submitted to a local application of 0.010 g of butyrospermol three times weekly is determined by comparison with untreated controls.

The resulting data are shown in Table III; the abbreviations have the following meanings:
IC : Index of cicatrization, percentage of the surface covered as compared to the original surface of the

wound:
M : percentage of mortality.

TABLE III

Day following the day of experimental injury	10th		20th	
	I.C.	M.	I.C.	M.
Controls (20 subjects)	49	20	72	40
Treated (20 subjects)	78	15	89	15

III - Bacteriostatic action:
Butyrospermol has bactericidal properties with respect to acid-resistant bacilli, especially with respect to Koch bacillus and to Hansen bacillus in vitro.

Following a first phase of normal, sometimes accelerated, developement, a solution of butyrospermol causes a marked inhibition of cultures of Koch bacilli or of Hansen bacilli from a 0.015 g/ml concentration.

The hormonal properties of butyrospermol may be advantageously used in the following cases: secondary suprarenal insufficiency, Addison's disease or ovarian insufficiency. Butyrospermol is also effective as an adjuvant of the follicular steroids.

Butyrospermol may also be used for its cicatrizing properties with respect to wounds either on local or on general adolinistration.

Finally, butyrospermol is active against the bacilli of leprosy and tuberculosis of the slain, as against all the Grampositive cocci (staphylocoeci and streptococci).

For these various uses, butyrospermol may be incorporated into pharmaceutical compositions in association with a pharmaceutically administrable vehicle, which, when liquid, is sterile.

This vehicle depends upon the method of administration, this generally being systemic, although topical application is also possible.

When administered systemically, butyrospermol may be given in a dose of 0.100 to 0.500 g daily, either orally in association with a solid vehicle, or parenterally in association with a sterile liquid vehicle.

Thus, for oral administration, tablets each containing for example, 0.050 g of the active compound in association with the usual excipients will be advantageously used.

Compositions for parenteral administration may be made up in two separate parts, to be mixed immediately before use, namely: - a sterilized bottle containing for example 0.050 g of sterile butyrospermol, - an ampoule of 1 ml of sterile solvent.

For topical application, the butyrospermol may be made into balm and ointment formulations by incorporating it into the usual pasty vehicles.

WHAT WE CLAIM IS:.
1. A therapeutic composition comprising as active compound in association with a pharmaceutically administrable solid, semi-solid or sterile liquid vehicle, butyrospermol 3, - hydroxy ~ (131on 14f3, 17,B H) - lanosta 7,24diene having the formula illustrated in the drawing accompanying the Provisional Spceification.

2. A composition as claimed in claim 1, in tablet form, the vehicle being a pharmaceutically acceptable inert solid.

3. A composition as claimed in claim 1, characterized for injection formulated as two parts to be mixed

extemporaneously and comprising: - a sterile bottle containing sterile butyro-spermol.

- an ampoule of sterile solvent.

4. A composition as claimed in claim 1, formulated as a balm or ointment, the vehicle

WARNING end of DESC field may overlap start of CLMS **.

WEST

Generate Collection

L1: Entry 16 of 32 File: USPT Apr 28, 1987

DOCUMENT-IDENTIFIER: US 4661343 A
TITLE: Aqueous or anhydrous cosmetic preparation containing a fatty phase consisting essentially of karite oil

BSPR:
Karite butter itself comes from the fruit (almonds) of the tree (Butyrospermum Parkii) found in Mali, Sudan, Senegal and Gabon. The almond contains from 45 to 55% fat which is extracted and refined.

L1: Entry 17 of 32 File: USPT Jun 10, 1986

DOCUMENT-IDENTIFIER: US 4594194 A
TITLE: Fat fractionation

BSPR:
The vegetable fats capable of being fractionated in accordance with the invention are those which show a crystalline polymorphism around ambient temperature, i.e. between 20.degree. and 35.degree. C. Vegetable fats such as these, which are known generically as vegetable tallows, include shea butter (karite, Butyrospermum parkii or Bassia parkii), sal butter (Shorea robusta), Borneo tallow (Shorea stenoptera), kokum butter (Garcinia indica), mango kernel butter (Mangifera indica), mowrah fat (Madhuca latifolia and Madhuca longifolia), palm oil (Elaeis guineensis), etc.

L1: Entry 18 of 32 File: USPT Jun 5, 1979

DOCUMENT-IDENTIFIER: US 4157405 A
TITLE: Cocoa butter substitutes and their preparation

DEPR:
Blends of the sal fat of this invention and one or more of oils and fats such as palm oil, palm olein, a middle melting fraction of palm oil, mowrah fat (seed fat of Madhuca longifolia) and its fractions, shea fat (see fat of Butyrospermum parkii) and its fractions, kokum butter (seed fat of Garcinia indica) and its fractions, mango kernel (seed fat of Mangifera indica) and its fractions are suitable for use as cocoa butter substitutes of better quality. The above-mentioned middle melting fraction of palm oil is the fraction which is obtained by removing lower and higher melting fractions from palm oil by a method of fractionating oils and fats. The above-mentioned palm olein is the fraction which is obtained by removing a higher melting fraction from palm oil by a method of fractionating oils and fats. As above-mentioned, a cocoa butter substitute composition of better quality having improved characteristic properties of the refined sal fat can be obtained by mixing the above-mentioned oils and fats with the refined sal fat. Specifically, a cocoa butter substitute composition comprising 80-95% by weight of the refined sal fat and 5-20% by weight of refined palm oil having an iodine value of 47-54 has a very good quality and improved characteristic properties as a cocoa butter substitute. A cocoa butter substitute composition can be obtained by mixing the above-mentioned oils and fats with the refined sal fat and also by refining a mixture of crude sal fat and crude oils and fats such as crude palm oil, crude mowrah fat, crude shea fat, crude kokum fat, crude mango kernel oil, and crude illipe fat under the condition of changing crude sal fat into refined sal fat according to the present invention.

WEST

Generate Collection

L1: Entry 21 of 32 File: EPAB May 14, 1999

DOCUMENT-IDENTIFIER: WO 9922706 A1
TITLE: COSMETIC OR DERMOPHARMACEUTICAL COMPOSITIONS CONTAINING A PLANT EXTRACT OBTAINED FROM THE SHEA TREE OR BUTYROSPERMUM PARKII KOTSCHY FLOWER

FPAR:
The invention concerns cosmetic or dermophamaceutical compositions containing, in sufficient amount, a plant extract obtained from the shea tree or Butyrospermum parkii Kotschy flower. The active principles contained in the shea tree (Butyrospermum parkii Kotschy) flower can be obtained by two different methods: extraction or distillation of dried flowers to obtain what is known as hydrolate or floral water. The cosmetic or dermopharmaceutical compositions containing one or other of said plant extracts are advantageously used for skin dryness, dermatitis and dermatosis, eczema, sunburns and burns, and when refreshing, deodorising, astringent, toning, healing, anti-crack, anti-wrinkle activities are required and for oral hygiene.

WEST

Generate Collection

L1: Entry 23 of 32 File: DWPI Sep 6, 2000

DERWENT-ACC-NO: 2001-150017
DERWENT-WEEK: 200116

TITLE: Cosmetic composition, e.g. skincare composition, for preventing premature ageing and dryness, comprises squalene, mixed triester, natural fatty acid glyceride, bridged malonamide, and a carrier< br>
ABTX:
DETAILED DESCRIPTION - A cosmetic composition comprises (wt.%) (a) a squalene (0.1-7.5) derived from a plant source, (b) a mixed triester (0.1-15) formed from the reaction of glycerin, caprylic, and capric acids, (c) a natural fatty acid glyceride (0.1-10) which is obtained from the nuts of the karite or shea butter tree (Butyrospermum Parkii), (d) a bridged malonamide (0.05-2.75) having a molecular weight of greater than 450, and (e) a cosmetically acceptable carrier, of the total weight of the composition, in which the combined amounts of the ingredients is not more than 17 wt.% of the total composition.

WEST

Generate Collection

L1: Entry 25 of 32 File: DWPI May 24, 1999

DERWENT-ACC-NO: 1999-290516
DERWENT-WEEK: 199940

TITLE: Cosmetic and dermopharmaceutical compositions useful for treating dermatitis, dermatoses, eczema solar erythema and burns

ABTX:
NOVELTY - Cosmetic and dermopharmaceutical compositions containing an extract obtained from the flowers of the karite or shea tree, Butyrospermum parkii Kotschy, Mangifolia, Poissoni, or Nilotica, are new.

L1: Entry 28 of 32 File: DWPI Nov 17, 1992

DERWENT-ACC-NO: 1992-430043
DERWENT-WEEK: 199252

TITLE: Cosmetic material compsn., imparting plain touch to hair and skin - contains liq. shea butter or butyrospermum parkii seeds

ABTX:
Compsn. contains the liq. shea butter of a clouding pt. of up to 20.0 deg. C which is obtd. from shea butter, or the fat from the seeds of Butyrospermum parkii. The liq. shea butter is sepd. e.g by vacuum distn. or solvent fractional crystallisation.

WEST

Generate Collection

Search Results - Record(s) 1 through 30 of 32 returned.

☐ 1. Document ID: US 6218345 B1

L1: Entry 1 of 32 File: USPT Apr 17, 2001

US-PAT-NO: 6218345
DOCUMENT-IDENTIFIER: US 6218345 B1

TITLE: Cleansing compositions

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brooks; Alan	Slough	N/A	N/A	GBX
Du Reau; Charles Marie Alain	London	N/A	N/A	GBX

US-CL-CURRENT: 510/123; 424/70.12, 424/70.13, 424/70.16, 424/70.19, 424/70.21, 424/70.22, 424/70.24, 424/70.31, 510/124 , 510/125, 510/127, 510/137, 510/138, 510/139, 510/159, 510/473, 510/499, 510/502, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6217874 B1

L1: Entry 2 of 32 File: USPT Apr 17, 2001

US-PAT-NO: 6217874
DOCUMENT-IDENTIFIER: US 6217874 B1

TITLE: Fat compositions and their use in cosmetic and pharmaceutical emulsion products

DATE-ISSUED: April 17, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Johannsen; Frank	Viby	N/A	N/A	DKX

US-CL-CURRENT: 424/727; 424/750, 424/755, 424/757, 424/764, 424/766, 424/776, 514/557

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6191083 B1

L1: Entry 3 of 32 File: USPT Feb 20, 2001

US-PAT-NO: 6191083
DOCUMENT-IDENTIFIER: US 6191083 B1

TITLE: Cleansing compositions

DATE-ISSUED: February 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brooks; Alan	Slough	N/A	N/A	GBX
Du Reau; Charles Marie Alain	London	N/A	N/A	GBX

US-CL-CURRENT: 510/124; 424/70.12, 424/70.13, 424/70.16, 424/70.19, 424/70.22, 424/70.24, 424/70.31, 510/123, 510/125, 510/127, 510/137, 510/138, 510/139, 510/159, 510/473, 510/499, 510/502, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 4. Document ID: US 6133212 A

L1: Entry 4 of 32 File: USPT Oct 17, 2000

US-PAT-NO: 6133212
DOCUMENT-IDENTIFIER: US 6133212 A

TITLE: Cleansing compositions

DATE-ISSUED: October 17, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Russell Phillip	Egham	N/A	N/A	GBX
Leahy; Christopher David	Kew Richmond	N/A	N/A	GBX
Holloway; Sara Louise	Surrey	N/A	N/A	GBX
Du Reau; Charles Marie	London	N/A	N/A	GBX

US-CL-CURRENT: 510/159; 510/130, 510/158, 510/493, 510/499, 510/504, 510/505, 510/506

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 5. Document ID: US 6074996 A

L1: Entry 5 of 32 File: USPT Jun 13, 2000

US-PAT-NO: 6074996
DOCUMENT-IDENTIFIER: US 6074996 A

TITLE: Liquid personal cleansing composition containing cationic polymeric skin conditioning agent

DATE-ISSUED: June 13, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Russell Phillip	Surrey	N/A	N/A	GBX
Green; Matthew Thomas	Middlesex	N/A	N/A	GBX
Leahy; Christopher David	Surrey	N/A	N/A	GBX

US-CL-CURRENT: 510/125; 424/70.12, 424/70.13, 424/70.16, 424/70.19, 424/70.21, 424/70.22, 424/70.24, 424/70.31, 510/123 , 510/124, 510/126, 510/127, 510/137, 510/138, 510/158, 510/159, 510/504, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 6. Document ID: US 6022381 A

L1: Entry 6 of 32 File: USPT Feb 8, 2000

US-PAT-NO: 6022381
DOCUMENT-IDENTIFIER: US 6022381 A

TITLE: Oxidative hair coloring compositions which contain a preformed organic peroxyacid oxidizing agent

DATE-ISSUED: February 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dias; Louis Carlos	Surrey	N/A	N/A	GBX
Pullan; Rowena Juliet Flux	Surrey	N/A	N/A	GBX
Sanger; Alison Jane	Farnborough	N/A	N/A	GBX

US-CL-CURRENT: 8/406; 8/401, 8/431

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 7. Document ID: US 6004915 A

L1: Entry 7 of 32 File: USPT Dec 21, 1999

US-PAT-NO: 6004915
DOCUMENT-IDENTIFIER: US 6004915 A

TITLE: Cleansing compositions

DATE-ISSUED: December 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Russell Phillip	Egham	N/A	N/A	GBX
Phipps; Nicola Jacqueline	Green Lane	N/A	N/A	GBX

US-CL-CURRENT: 510/135; 510/119, 510/130, 510/417, 510/422, 510/426, 510/427

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 8. Document ID: US 6004355 A

L1: Entry 8 of 32 File: USPT Dec 21, 1999

US-PAT-NO: 6004355
DOCUMENT-IDENTIFIER: US 6004355 A

TITLE: Hair coloring compositions comprising a peroxygen oxidizing agent, an organic peroxyacid precursor, and oxidative hair coloring agents

DATE-ISSUED: December 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dias; Louis Carlos	Surrey	N/A	N/A	GBX
Pullan; Rowena Juliet Flux	Surrey	N/A	N/A	GBX
Sanger; Alison Jane	Farnborough	N/A	N/A	GBX

US-CL-CURRENT: 8/406; 8/401, 8/431

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 9. Document ID: US 5985809 A

L1: Entry 9 of 32 File: USPT Nov 16, 1999

US-PAT-NO: 5985809
DOCUMENT-IDENTIFIER: US 5985809 A

TITLE: Aqueous personal cleansing compositions comprising specific nonocclusive liquid polyol fatty acid polyester

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frankenbach; Gayle Marie	Cincinnati	OH	N/A	N/A
Phipps; Nicola Jacqueline	Surrey	N/A	N/A	GBX
Richardson; Wendy Victoria J.	Middlesex	N/A	N/A	GBX

US-CL-CURRENT: 510/159; 510/119, 510/121, 510/122, 510/135, 510/136, 510/137, 510/138, 510/158, 510/417, 510/427, 510/433, 510/470, 510/502, 510/507

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 10. Document ID: US 5968491 A

L1: Entry 10 of 32 File: USPT Oct 19, 1999

US-PAT-NO: 5968491
DOCUMENT-IDENTIFIER: US 5968491 A

TITLE: Detergent composition comprising clay and polysaccharide gum stabilizing agents

DATE-ISSUED: October 19, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Richardson; Wendy Victoria Jane	Middlesex	N/A	N/A	GBX

US-CL-CURRENT: 424/70.1; 424/401, 424/70.21, 424/70.22, 510/101, 510/121, 510/159, 510/507

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 11. Document ID: US 5910472 A

L1: Entry 11 of 32 File: USPT Jun 8, 1999

US-PAT-NO: 5910472
DOCUMENT-IDENTIFIER: US 5910472 A

TITLE: Cleansing compositions

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Russell Phillip	Egham	N/A	N/A	GBX
Green; Matthew Thomas	Teddington	N/A	N/A	GBX
Leahy; Christopher David	Kew	N/A	N/A	GBX
Papadimitriou; Eleni	Putney	N/A	N/A	GBX

US-CL-CURRENT: 510/124; 510/123, 510/125, 510/127, 510/137, 510/138, 510/158, 510/159, 510/473, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 12. Document ID: US 5905062 A

L1: Entry 12 of 32 File: USPT May 18, 1999

US-PAT-NO: 5905062
DOCUMENT-IDENTIFIER: US 5905062 A

TITLE: Cleansing compositions technical field

DATE-ISSUED: May 18, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Elliott; Russell Phillip	Egham	N/A	N/A	GBX
Green; Matthew Thomas	Teddington	N/A	N/A	GBX
Leahy; Christopher David	Kew Richmond	N/A	N/A	GBX
Papadimitriou; Eleni	Putney	N/A	N/A	GBX

US-CL-CURRENT: 510/124; 424/70.12, 424/70.13, 424/70.15, 424/70.16, 424/70.19, 424/70.21, 424/70.22, 424/70.31, 510/123 , 510/125, 510/127, 510/137, 510/138, 510/159, 510/473, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 13. Document ID: US 5858342 A

L1: Entry 13 of 32 File: USPT Jan 12, 1999

US-PAT-NO: 5858342
DOCUMENT-IDENTIFIER: US 5858342 A

TITLE: Cleansing compositions

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Giret; Michel Joseph Marie	Camberley	N/A	N/A	GB3
Bellemain; Chantal Marie	Staines	N/A	N/A	GB3

US-CL-CURRENT: 424/70.19; 514/784, 514/846

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

---

☐ 14. Document ID: US 5660865 A

L1: Entry 14 of 32 File: USPT Aug 26, 1997

US-PAT-NO: 5660865
DOCUMENT-IDENTIFIER: US 5660865 A

TITLE: Surface treatment composition

DATE-ISSUED: August 26, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pedersen; Arne	Hinnerup	N/A	N/A	DKX
Johannsen; Frank	Viby J	N/A	N/A	DKX

US-CL-CURRENT: 426/99; 426/307, 426/601

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

---

☐ 15. Document ID: US 5262154 A

L1: Entry 15 of 32 File: USPT Nov 16, 1993

US-PAT-NO: 5262154
DOCUMENT-IDENTIFIER: US 5262154 A

TITLE: Shaving preparation

DATE-ISSUED: November 16, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wendel; Otto W.	Houston	TX	N/A	N/A
Chang; Pauley	Houston	TX	N/A	N/A

US-CL-CURRENT: 424/73; 424/47, 514/938, 514/944, 516/102, 516/67, 516/DIG.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 16. Document ID: US 4661343 A

L1: Entry 16 of 32 File: USPT Apr 28, 1987

US-PAT-NO: 4661343
DOCUMENT-IDENTIFIER: US 4661343 A

TITLE: Aqueous or anhydrous cosmetic preparation containing a fatty phase consisting essentially of karite oil

DATE-ISSUED: April 28, 1987

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zabotto; Arlette	Paris	N/A	N/A	FRX
Griat; Jacqueline	Ablon	N/A	N/A	FRX
Bracco; Umberto	La Tour de Peilz	N/A	N/A	SEX

US-CL-CURRENT: 424/59; 424/60, 424/63, 424/DIG.5, 514/844, 514/845, 514/846, 514/847, 514/937, 514/938, 514/969

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 17. Document ID: US 4594194 A

L1: Entry 17 of 32 File: USPT Jun 10, 1986

US-PAT-NO: 4594194
DOCUMENT-IDENTIFIER: US 4594194 A

TITLE: Fat fractionation

DATE-ISSUED: June 10, 1986

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dieffenbacher; Albrecht	Saint-Legier	N/A	N/A	CHX

US-CL-CURRENT: 554/211

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 18. Document ID: US 4157405 A

L1: Entry 18 of 32 File: USPT Jun 5, 1979

US-PAT-NO: 4157405
DOCUMENT-IDENTIFIER: US 4157405 A

TITLE: Cocoa butter substitutes and their preparation

DATE-ISSUED: June 5, 1979

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yasuda; Nozomi	Tokyo	N/A	N/A	JPX
Terada; Kimio	Tokyo	N/A	N/A	JPX
Itagaki; Kazuo	Tokyo	N/A	N/A	JPX
Toyoshima; Yasuo	Tokyo	N/A	N/A	JPX
Maruzeni; Shouji	Tokyo	N/A	N/A	JPX
Itoh; Tadasu	Tokyo	N/A	N/A	JPX
Yokobori; Hideo	Tokyo	N/A	N/A	JPX
Satoh; Susumu	Tokyo	N/A	N/A	JPX

US-CL-CURRENT: 426/607; 554/190, 554/191, 554/205, 554/207

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw Desc | Image

---

☐ 19. Document ID: JP 04327517 A

L1: Entry 19 of 32 File: JPAB Nov 17, 1992

PUB-NO: JP404327517A
DOCUMENT-IDENTIFIER: JP 04327517 A
TITLE: COSMETIC COMPOSITION

PUBN-DATE: November 17, 1992

INVENTOR-INFORMATION:

NAME	COUNTRY
KORESAWA, TAKESHI	

INT-CL (IPC): A61K 7/00; A61K 7/06; A61K 7/42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw Desc | Image

---

☐ 20. Document ID: JP 02191210 A

L1: Entry 20 of 32 File: JPAB Jul 27, 1990

PUB-NO: JP402191210A
DOCUMENT-IDENTIFIER: JP 02191210 A
TITLE: COSMETIC COMPOSITION

PUBN-DATE: July 27, 1990

INVENTOR-INFORMATION:

NAME	COUNTRY
HASEGAWA, MOTOO	
KORESAWA, TAKESHI	

INT-CL (IPC): A61K 7/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 21. Document ID: WO 9922706 A1

L1: Entry 21 of 32 File: EPAB May 14, 1999

PUB-NO: WO009922706A1
DOCUMENT-IDENTIFIER: WO 9922706 A1
TITLE: COSMETIC OR DERMOPHARMACEUTICAL COMPOSITIONS CONTAINING A PLANT EXTRACT OBTAINED FROM THE SHEA TREE OR BUTYROSPERMUM PARKII KOTSCHY FLOWER

PUBN-DATE: May 14, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
LINTNER, KARL	FR

INT-CL (IPC): A61K 7/48
EUR-CL (EPC): A61K007/48

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 22. Document ID: AU 200058066 A, WO 200103712 A1

L1: Entry 22 of 32 File: DWPI Jan 30, 2001

DERWENT-ACC-NO: 2001-138255
DERWENT-WEEK: 200127

TITLE: Pharmaceutical or dietary composition comprising Butyrospermum parkii e.g. for immunomodulation or treating hypersensitivity or inflammatory conditions

INVENTOR: WEIDNER, M S

PRIORITY-DATA: 2000US-0190919 (March 21, 2000), 1999DK-0001003 (July 9, 1999), 1999DK-0001323 (September 16, 1999), 1999US-0154651 (September 20, 1999), 2000DK-0000434 (March 16, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200058066 A	January 30, 2001	N/A	000	A61K035/78
WO 200103712 A1	January 18, 2001	E	039	A61K035/78

INT-CL (IPC): A61K 35/78; A61P 1/04; A61P 13/08; A61P 17/00; A61P 17/06; A61P 19/02; A61P 29/00; A61P 37/00; A61P 37/02

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 23. Document ID: IE 81286 B3

L1: Entry 23 of 32 File: DWPI Sep 6, 2000

DERWENT-ACC-NO: 2001-150017
DERWENT-WEEK: 200116

TITLE: Cosmetic composition, e.g. skincare composition, for preventing premature ageing and dryness, comprises squalene, mixed triester, natural fatty acid glyceride, bridged malonamide, and a carrier

INVENTOR: DOYLE, M; FERGUSON, J

PRIORITY-DATA: 1998IE-0000410 (May 29, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
IE 81286 B3	September 6, 2000	N/A	012	A61K007/48

INT-CL (IPC): A61K 7/48

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 24. Document ID: AU 9939367 A, WO 9963963 A1, FR 2779645 A1

L1: Entry 24 of 32 File: DWPI Dec 30, 1999

DERWENT-ACC-NO: 2000-223543
DERWENT-WEEK: 200022

TITLE: Cosmetic composition for soothing the skin and reducing inflammation, lines and wrinkles, by combating effects of free radicals, contains green coffee and shea butter extracts

INVENTOR: LINTNER, K

PRIORITY-DATA: 1998FR-0007406 (June 11, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9939367 A	December 30, 1999	N/A	000	A61K007/48
WO 9963963 A1	December 16, 1999	F	013	A61K007/48
FR 2779645 A1	December 17, 1999	N/A	000	A61K007/48

INT-CL (IPC): A61K 7/48; A61K 35/78

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 25. Document ID: AU 9894484 A, FR 2770400 A1, WO 9922706 A1

L1: Entry 25 of 32 File: DWPI May 24, 1999

DERWENT-ACC-NO: 1999-290516
DERWENT-WEEK: 199940

TITLE: Cosmetic and dermopharmaceutical compositions useful for treating dermatitis, dermatoses, eczema solar erythema and burns

INVENTOR: LINTNER, K

PRIORITY-DATA: 1997FR-0013755 (October 30, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9894484 A	May 24, 1999	N/A	000	A61K007/48
FR 2770400 A1	May 7, 1999	N/A	009	A61K007/48
WO 9922706 A1	May 14, 1999	F	000	A61K007/48

INT-CL (IPC): A61K 7/48; A61K 35/78

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 26. Document ID: JP 07025741 A

L1: Entry 26 of 32 File: DWPI Jan 27, 1995

DERWENT-ACC-NO: 1995-101776
DERWENT-WEEK: 199514

TITLE: External skin preparation useful for skin-whitening cosmetics - contains kojic acid and/or its derivs., and at least one of millet, orange, avocado, Chlorella, peach kernel etc.

PRIORITY-DATA: 1993JP-0174577 (July 14, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 07025741 A	January 27, 1995	N/A	014	A61K007/48

INT-CL (IPC): A61K 7/42; A61K 7/48; A61K 7/50; A61K 9/06; A61K 9/08; A61K 9/70; A61K 31/12; A61K 35/78; A61K 47/46

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Clip Img | Image

---

☐ 27. Document ID: JP 3113705 B2, JP 05039225 A

L1: Entry 27 of 32 File: DWPI Dec 4, 2000

DERWENT-ACC-NO: 1993-096739
DERWENT-WEEK: 200065

TITLE: Blood circulation accelerator for application to skin - contains fat and oil from seed of Butyrospermum parkil as effective component

PRIORITY-DATA: 1991JP-0196708 (August 6, 1991)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 3113705 B2	December 4, 2000	N/A	004	A61K035/78
JP 05039225 A	February 19, 1993	N/A	004	A61K035/78

INT-CL (IPC): A61K 7/00; A61K 9/06; A61K 35/78; A61P 9/00; A61P 17/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 28. Document ID: JP 04327517 A

L1: Entry 28 of 32 File: DWPI Nov 17, 1992

DERWENT-ACC-NO: 1992-430043
DERWENT-WEEK: 199252

TITLE: Cosmetic material compsn., imparting plain touch to hair and skin - contains liq. shea butter or butyrospermum parkii seeds

PRIORITY-DATA: 1991JP-0122308 (April 23, 1991)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 04327517 A	November 17, 1992	N/A	004	A61K007/00

INT-CL (IPC): A61K 7/06; A61K 7/42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 29. Document ID: JP 02191210 A

L1: Entry 29 of 32 File: DWPI Jul 27, 1990

DERWENT-ACC-NO: 1990-271369
DERWENT-WEEK: 199036

TITLE: Surfactant cosmetic compsn. - includes gum component produced from seed of butyrospermum parkii

PRIORITY-DATA: 1989JP-0009199 (January 18, 1989)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 02191210 A	July 27, 1990	N/A	000	N/A

INT-CL (IPC): A61K 7/00

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 30. Document ID: NL 7500595 A, FR 2259072 A, GB 1457342 A

L1: Entry 30 of 32 File: DWPI Jul 29, 1975

DERWENT-ACC-NO: 1975-55059W
DERWENT-WEEK: 197533

TITLE: Culture medium for mushrooms - contg. manure and ground butter-tree nuts

PRIORITY-DATA: 1974GB-0003688 (January 25, 1974)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
NL 7500595 A	July 29, 1975	N/A	000	N/A
FR 2259072 A	September 26, 1975	N/A	000	N/A
GB 1457342 A	November 30, 1976	N/A	000	N/A

INT-CL (IPC): A01G 1/04; C05F 3/00; C05F 11/08

WEST

Generate Collection

L1: Entry 31 of 32 File: DWPI

DERWENT-ACC-NO: 1971-09123S
DERWENT-WEEK: 197105

TITLE: Shea gum use

ABTX:
Shea butter from Butyrospermum parkii Kotschy is dissolved in hot org. solvent and cooled to 28-30 degrees C to ppte a gum. The system is reheated to aggregate the gum, and then sepd. and treated in the cold solvent, followed by utilisation as a material for chewing gum base. The gum is readily oxidised because of being rich in unsatd. grps. but it can be stored without change when it is mixed with dibutylhydroxy-toluene.

WEST

End of Result Set

☐ Generate Collection

L1: Entry 32 of 32 File: DWPI

DERWENT-ACC-NO: 1966-08443F
DERWENT-WEEK: 196800

TITLE: Butyrospermol compn

ABTX:
(I) obtd. from kernels of Butyrospermum Parkii (karite) or latex of breadfruit tree Artocarpus integrifolia by extrn. with CCl4, treatment with NaOH in MeOH then chromagraphy to give mixt. contng. (I), beta-amyrin and parkeol-suitable for therapeutic use or (I) sepd. by acetylation, chromatography and hydrolysis.

WEST

Generate Collection

Search Results - Record(s) 31 through 32 of 32 returned.

☐ 31. Document ID: JP 71004149 B

L1: Entry 31 of 32 File: DWPI

DERWENT-ACC-NO: 1971-09123S
DERWENT-WEEK: 197105

TITLE: Shea gum use

PRIORITY-DATA: 1967JP-0031071 (May 16, 1967)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 71004149 B		N/A	000	N/A

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 32. Document ID: GB 932662 A

L1: Entry 32 of 32 File: DWPI

DERWENT-ACC-NO: 1966-08443F
DERWENT-WEEK: 196800

TITLE: Butyrospermol compn

PRIORITY-DATA: 1960GB-0013221 (April 13, 1960)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
GB 932662 A		N/A	000	N/A

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

Generate Collection

Terms	Documents
butyrospermum	32

Display 30 Documents, starting with Document: 32

Display Format: CIT Change Format

WEST

Generate Collection

Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: AU 200058066 A, WO 200103712 A1

L4: Entry 1 of 1 File: DWPI Jan 30, 2001

DERWENT-ACC-NO: 2001-138255
DERWENT-WEEK: 200127

TITLE: Pharmaceutical or dietary composition comprising Butyrospermum parkii e.g. for immunomodulation or treating hypersensitivity or inflammatory conditions

INVENTOR: WEIDNER, M S

PRIORITY-DATA: 2000US-0190919 (March 21, 2000), 1999DK-0001003 (July 9, 1999), 1999DK-0001323 (September 16, 1999), 1999US-0154651 (September 20, 1999), 2000DK-0000434 (March 16, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200058066 A	January 30, 2001	N/A	000	A61K035/78
WO 200103712 A1	January 18, 2001	E	039	A61K035/78

INT-CL (IPC): A61K 35/78; A61P 1/04; A61P 13/08; A61P 17/00; A61P 17/06; A61P 19/02; A61P 29/00; A61P 37/00; A61P 37/02

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

Generate Collection

Terms	Documents
l3 and butyrospermol	1

Display 30 Documents, starting with Document: 1

Display Format: CIT Change Format

WEST

Generate Collection

L3: Entry 8 of 13 File: USPT Dec 31, 1996

DOCUMENT-IDENTIFIER: US 5589619 A
TITLE: Process and composition for increasing squalene and sterol accumulation in higher plants

DEPR:
Pentacyclic triterpenoids are sterol compounds having a fifth ring formed from cyclization of the steroidal 17-position side chain. Examples of these compounds include alpha-amyrin, beta-amyrin and lupeol. Although these compounds are found in a wide variety of plants, they are usually present in only trace amounts. These compounds and their conjugates (e.g. saponins) are reported to have medicinal and insecticidal properties.

WEST

Generate Collection

Search Results - Record(s) 1 through 13 of 13 returned.

☐ 1. Document ID: US 6207826 B1

L3: Entry 1 of 13 File: USPT Mar 27, 2001

US-PAT-NO: 6207826
DOCUMENT-IDENTIFIER: US 6207826 B1

TITLE: Macrocyclic compounds having nitrogen-containing linkages

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cook; Phillip Dan	San Macros	CA	N/A	N/A
Guinosso; Charles J.	Vista	CA	N/A	N/A
Kung; Pei-Pei	Carlsbad	CA	N/A	N/A
Fraser; Allister S.	Carlsbad	CA	N/A	N/A

US-CL-CURRENT: 540/472; 540/473, 540/474

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 6197965 B1

L3: Entry 2 of 13 File: USPT Mar 6, 2001

US-PAT-NO: 6197965
DOCUMENT-IDENTIFIER: US 6197965 B1

TITLE: Compounds having a plurality of nitrogenous substituents

DATE-ISSUED: March 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cook; P. Dan	Vista	CA	N/A	N/A
An; Haoyun	Encinitas	CA	N/A	N/A

US-CL-CURRENT: 546/334; 546/271.4, 546/272.4, 546/278.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 6191273 B1

L3: Entry 3 of 13 File: USPT Feb 20, 2001

US-PAT-NO: 6191273
DOCUMENT-IDENTIFIER: US 6191273 B1

TITLE: Substituted cyclic compounds and mixtures comprising same

DATE-ISSUED: February 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cook; Phillip Dan	Lake San Marcos	CA	N/A	N/A
An; Haoyun	Encinitas	CA	N/A	N/A
Haly; Becky	La Mesa	CA	N/A	N/A
Wang; Tingmin	Carlsbad	CA	N/A	N/A

US-CL-CURRENT: 540/472

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 4. Document ID: US 6107482 A

L3: Entry 4 of 13 File: USPT Aug 22, 2000

US-PAT-NO: 6107482
DOCUMENT-IDENTIFIER: US 6107482 A

TITLE: Nitrogenous macrocyclic compounds

DATE-ISSUED: August 22, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cook; Phillip Dan	Escondido	CA	N/A	N/A
An; Haoyun	Encinitas	CA	N/A	N/A
Guinosso; Charles J.	Vista	CA	N/A	N/A
Kung; Pei-Pei	Leucadia	CA	N/A	N/A
Fraser; Allister S.	San Marcos	CA	N/A	N/A

US-CL-CURRENT: 540/472; 540/455, 540/469

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 5. Document ID: US 6077954 A

L3: Entry 5 of 13 File: USPT Jun 20, 2000

US-PAT-NO: 6077954
DOCUMENT-IDENTIFIER: US 6077954 A

TITLE: Substituted heterocyclic compounds

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cook; P. Dan	Vista	CA	N/A	N/A
An; Haoyun	Encinitas	CA	N/A	N/A

US-CL-CURRENT: 544/353; 544/162, 544/182, 544/301, 544/333, 544/335, 544/336, 544/354, 544/405, 546/271.4, 546/272.4, 546/278.1, 546/334, 548/264.4, 548/267.8, 548/950, 548/967, 549/426, 549/492, 549/74, 549/75

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 6. Document ID: US 5981420 A

L3: Entry 6 of 13 File: USPT Nov 9, 1999

US-PAT-NO: 5981420
DOCUMENT-IDENTIFIER: US 5981420 A

TITLE: Oxidation catalytic system and oxidation process

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nakano; Tatsuya	Himeji	N/A	N/A	JPX
Ishii; Yasutaka	Takatsuki	N/A	N/A	JPX

US-CL-CURRENT: 502/155; 502/162, 502/204, 502/213, 562/409, 562/549, 568/357, 568/431, 568/836

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 7. Document ID: US 5958821 A

L3: Entry 7 of 13 File: USPT Sep 28, 1999

US-PAT-NO: 5958821
DOCUMENT-IDENTIFIER: US 5958821 A

TITLE: Oxidation catalytic system and oxidation process using the same

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ishii; Yasutaka	Takatsuki	N/A	N/A	JPX
Nakano; Tatsuya	Himeji	N/A	N/A	JPX

US-CL-CURRENT: 502/167; 502/152, 548/545, 548/549, 548/551, 548/552

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 8. Document ID: US 5589619 A

L3: Entry 8 of 13 File: USPT Dec 31, 1996

US-PAT-NO: 5589619
DOCUMENT-IDENTIFIER: US 5589619 A

TITLE: Process and composition for increasing squalene and sterol accumulation in higher plants

DATE-ISSUED: December 31, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chappell; Joseph	Lexington	KY	N/A	N/A
Saunders; Court A.	Clarendon Hills	IL	N/A	N/A
Wolf; Fred R.	Naperville	IL	N/A	N/A

US-CL-CURRENT: 800/278; 435/69.1, 800/294, 800/302, 800/312, 800/314, 800/317.3, 800/317.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 9. Document ID: US 5349126 A

L3: Entry 9 of 13 File: USPT Sep 20, 1994

US-PAT-NO: 5349126
DOCUMENT-IDENTIFIER: US 5349126 A

TITLE: Process and composition for increasing squalene and sterol accumulation in higher plants

DATE-ISSUED: September 20, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chappell; Joseph	Lexington	KY	N/A	N/A
Saunders; Court A.	Clarendon Hills	IL	N/A	N/A
Wolf; Fred R.	Naperville	IL	N/A	N/A

US-CL-CURRENT: 800/265; 435/69.1, 435/70.1, 800/261, 800/302, 800/312, 800/314, 800/317.3, 800/317.4, 800/320.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 10. Document ID: US 4808574 A

L3: Entry 10 of 13 File: USPT Feb 28, 1989

US-PAT-NO: 4808574
DOCUMENT-IDENTIFIER: US 4808574 A

TITLE: Composition inhibiting pathological addiction to alcohol

DATE-ISSUED: February 28, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brekhman; Izrail I.	Vladivostok	N/A	N/A	SUX
Bulanov; Alexandr E.	Vladivostok	N/A	N/A	SUX
Polozhentseva; Mira I.	Vladivostok	N/A	N/A	SUX
Mudzhiri; Levan A.	Tbilisi	N/A	N/A	SUX
Alkhazashvili; Gia G.	Tbilisi	N/A	N/A	SUX
Kalatozishvili; Elena I.	Tbilisi	N/A	N/A	SUX
Dardymov; Igor V.	Vladivostok	N/A	N/A	SUX
Bezdetko; Gennady N.	Vladivostok	N/A	N/A	SUX
Khasina; Eleonora I.	Vladivostok	N/A	N/A	SUX

US-CL-CURRENT: 514/23; 426/11, 426/15, 514/811

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 11. Document ID: US 4515781 A

L3: Entry 11 of 13 File: USPT May 7, 1985

US-PAT-NO: 4515781
DOCUMENT-IDENTIFIER: US 4515781 A

TITLE: 2',5'-Riboadenylate-morpholinoadenylate nucleotides

DATE-ISSUED: May 7, 1985

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Torrence; Paul F.	Gaithersberg	MD	N/A	N/A
Johnston; Margaret I.	Washington	DC	N/A	N/A
Imai; Jiro	Kensington	MD	N/A	N/A

US-CL-CURRENT: 514/44; 514/47, 514/48, 536/25.2, 536/26.21, 536/26.26

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 12. Document ID: US 4220588 A

L3: Entry 12 of 13 File: USPT Sep 2, 1980

US-PAT-NO: 4220588
DOCUMENT-IDENTIFIER: US 4220588 A

TITLE: Chemical processes

DATE-ISSUED: September 2, 1980

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barton; Derek H. R.	91190 Gif sur Yvette	N/A	N/A	FRX
Lester; David J.	91190 Gif sur Yvette	N/A	N/A	FRX
Ley; Steven V.	London	N/A	N/A	GB2

US-CL-CURRENT: 540/19; 540/20, 552/505, 552/544

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

---

☐ 13. Document ID: AU 200058066 A, WO 200103712 A1

L3: Entry 13 of 13 File: DWPI Jan 30, 2001

DERWENT-ACC-NO: 2001-138255
DERWENT-WEEK: 200127

TITLE: Pharmaceutical or dietary composition comprising Butyrospermum parkii e.g. for immunomodulation or treating hypersensitivity or inflammatory conditions

INVENTOR: WEIDNER, M S

PRIORITY-DATA: 2000US-0190919 (March 21, 2000), 1999DK-0001003 (July 9, 1999), 1999DK-0001323 (September 16, 1999), 1999US-0154651 (September 20, 1999), 2000DK-0000434 (March 16, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200058066 A	January 30, 2001	N/A	000	A61K035/78
WO 200103712 A1	January 18, 2001	E	039	A61K035/78

INT-CL (IPC): A61K 35/78; A61P 1/04; A61P 13/08; A61P 17/00; A61P 17/06; A61P 19/02; A61P 29/00; A61P 37/00; A61P 37/02

Generate Collection

Terms	Documents
l2 and $$$amyrin	13

Display 30 Documents, starting with Document: 13

Display Format: CIT Change Format

WEST

Generate Collection

L2: Entry 20 of 44 File: USPT May 24, 1994

DOCUMENT-IDENTIFIER: US 5314877 A
TITLE: Water-soluble pentacyclic triterpene composition and method for producing the same

BSPR:
Triterpenes are classified into amylin, lupeol, nosterol and squalene groups. These are all compounds which are insoluble in water and soluble in oil, organic solvents or the like.

L2: Entry 29 of 44 File: USPT Apr 28, 1987

DOCUMENT-IDENTIFIER: US 4661343 A
TITLE: Aqueous or anhydrous cosmetic preparation containing a fatty phase consisting essentially of karite oil

BSPV:
Lupeol: 16+1%

WEST

Help | Logout | Interrupt

Main Menu | Search Form | Posting Counts | Show S Numbers | Edit S Numbers | Preferences

Search Results -

Terms	Documents
l3 and butyrospermol	1

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: `l3 and butyrospermol` Clear

Search History

Today's Date: 7/13/2001

DB Name	Query	Hit Count	Set Name
USPT,JPAB,EPAB,DWPI,TDBD	l3 and butyrospermol	1	L4
USPT,JPAB,EPAB,DWPI,TDBD	l2 and $$$amyrin	13	L3
USPT,JPAB,EPAB,DWPI,TDBD	lupeol$	44	L2
USPT,JPAB,EPAB,DWPI,TDBD	butyrospermum	32	L1

```
=> FIL MEDLINE, CAPLUS, SCISEARCH
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.15       0.15

FILE 'MEDLINE' ENTERED AT 10:11:56 ON 13 JUL 2001

FILE 'CAPLUS' ENTERED AT 10:11:56 ON 13 JUL 2001
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2001 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'SCISEARCH' ENTERED AT 10:11:56 ON 13 JUL 2001
COPYRIGHT (C) 2001 Institute for Scientific Information (ISI) (R)

=> s butryospermum(w)parkii
L1            0 BUTRYOSPERMUM(W) PARKII

=> s butyrospermum(w)parkii or butytpsermol
L2           43 BUTYROSPERMUM(W) PARKII OR BUTYTPSERMOL

=> s butyrospermum(w)parkii or butyrospermol
L3          187 BUTYROSPERMUM(W) PARKII OR BUTYROSPERMOL

=> s medicant or medicine or pharmaceutical?
L4       746675 MEDICANT OR MEDICINE OR PHARMACEUTICAL?

=> s inflam? or hypersensitiv? or pain or sensitive or aller?
L5      1861284 INFLAM? OR HYPERSENSITIV? OR PAIN OR SENSITIVE OR ALLER?

=> l3 and l4
L6            5 L3 AND L4

=> l6 and l5
L7            3 L6 AND L5

=> d 1-3
```

Patent Number: ☐ FR2779645
Publication date: 1999-12-17
Inventor(s): LINTNER KARL
Applicant(s):: SEDERMA SA (FR)
Requested Patent: ☐ WO9963963
Application Number: FR19980007406 19980611
Priority Number(s): FR19980007406 19980611
IPC Classification: A61K7/48 ; A61K35/78
EC Classification: A61K7/48W4, A61K35/78
Equivalents: AU3936799

Abstract

The invention concerns the product resulting from associating a plant extract obtained from Coffea arabica or Coffea canephora green coffee with shea butter obtained from the shea tree or Butyrospermum parkii Kotschy nut, and its use in cosmetic or dermopharmaceutical compositions. The product resulting from said association is used as such or in cosmetic or dermopharmaceutical compositions for preparing a medicine for healing, skin care and skin soothing effects, including treatment against the noxious effects of radical forms of oxygen such as, for example, skin inflammation, premature skin ageing or withering, occurrence of wrinkles, and for ensuring protection of the hair, scalp, nail and mucous membranes

D scription

CRANBERRY SEED OIL EXTRACT AND COMPOSITIONS CONTAINING COMPONENTS THEREOF

Related Information

This application claims priority to U. S. provisional Application No. 60/137,405, entitled"CRANBERRY SEED OIL EXTRACT AND COMPOSITIONS CONTAINING COMPONENTS THEREOF,"filed on June 1,1999, incorporated herein in its entirety by this reference. The contents of all patents, patent applications, and references cited throughout this specification are hereby incorporated by reference in their entireties.

Background of the Invention

For millennia, humankind has relied on plant derivatives for the prevention and treatment of a wide variety of aliments. For example, in China, various teas have been used as a crude medicine for over 4,000 years. And more recently, there has been considerable interest in taking advantage of various plant extracts as a source of health promoting substances such as, natural oxidants, flavonoids, and phenolic compounds. In part, this trend is due to a growing body of evidence demonstrating that some of these compounds have beneficial properties that may be advantageous in preventing or delaying, for example, the onset of cardiovascular disease.

Indeed, several studies have suggested that beneficial fatty acid and other plant derived compounds have desirable effects ranging from reducing lipid levels, lowering blood pressure, and regulating inflammatory disease. For example, barley has been shown to be particularly effective in lowering lipid levels in test animals (Quereshi et al., Lipids, 20: 817-24 (1985)). And in particular, a tocochromanol isolated from barley extract has been identified as an active compound suitable for treating hypercholesterolemia (Quereshi et al., J. Biol. Chem., 261: 10544-50 (1986)). Similarly, other tocochromanols, for example, y-tocotrienol and 6-tocotrienol have also been shown to reduce hypercholesterolemia in mammals (European patent application 421,419).

In general, hypercholesterolemia involves high serum cholesterol levels that are associated with a number of diseases including atherosclerosis, arteriosclerosis, and cardiovascular disease. In addition, high serum cholesterol levels are also seen in patients suffering from other diseases such as diabetes mellitus and familial hypercholesterolemia. While improvement of lipoprotein profiles and a decrease in total serum and low density lipoprotein cholesterol have been shown to slow the progression of such diseases, the exact link between hypercholesterolemia and, most notably, cardiovascular disease, has remained obscure. As a result, cardiovascular disease continues to remain a leading cause of death in the United States.

In part, the reason a cure for cardiovascular disease has remained elusive, is that the etiology of the disease may be the result of series of complex interactions involving genetic factors, lipoprotein metabolism, clotting functions, and even lifestyle choices (e. g, diet, exercise). Interestingly, populations consuming large amounts of cereal grains have a lower incidence of cardiovascular disease and lower cholesterol levels.

Studies looking at the beneficial properties of cereal diets have attributed these effects to naturally occurring tocochromanols. and these compounds have been found in a wide variety of plant sources (Quereshi et al., Am. J. Clin. Nutr., 53: 1021 S-6S (1991)).

As a class of compounds, tocochromanols include the tocopherols and the tocotrienols. Tocopherols, including 6-a-tocopherol are essentially the active ingredient in vitamin E and have been extensively studied. A number of beneficial properties have been attributed to the tocopherols such as reduced platelet aggregation and antioxidant functions (Niki et al., Annals of the New York Academy of Sciences, 570: 23-31 (1989); Fukuzawa et al., Annals of the New York Academy of Sciences, 570: 449-453 (1989)).

The tocotrienols have been less well studied although recent evidence suggests that these compounds may also be biologically active (see for example U. S. Patent 5,591,772 and Naturally occurring tocotrienols including a- ?-, y-, and 5- tocotrienol have been identified in and isolated from a variety of sources including, e. g., rice, rice bran, barley, coconut, and palm. These compounds exhibit varying degrees of hypercholesterolemic activity and have also been used as antithrombotic agents and

antioxidants.

Additional sources of tocopherols, tocotrienols, and other therapeutically beneficial compounds which can be used safely and effectively. for example, as a hypercholesterolemic, antithrombotic. antioxidizing, antiatherogenic, antiinflammatory, and immunoregulatory agents, would be of great benefit.

Summary of the Invention

The present invention provides isolated cranberry seed oil. a novel source of health promoting compounds (e. g., desirable fatty acids, tocochromanols) that are useful in a variety of therapeutic applications, for example, as a hypocholesterolemic, antithrombotic, antioxidizing, antiatherogenic, antiinflammatory. and immunoregulatory agents. In addition, the invention provides methods of efficiently extracting cranberry seed oil to a high level of purity from cranberry seeds, for example, such that the extract can be added to foodstuffs or used as a dietary supplement or a pharmaceutical composition.

Accordingly, in one aspect, the invention provides an isolated cranberry seed oil extract which is substantially free of impurities. The extract can contain, for example, a-tocopherol, y-tocopherol, b-tocopherol, (x-tocotrienol, y-tocotrienol, 6-tocotrienol, or a combination thereof. In another embodiment, the extract further comprises an exogenous flavonoid, tamoxifen, or a combination thereof. In a related embodiment, the flavonoid is a flavone, flavavone, isoflavone, or flavonol. In another embodiment, the extract further comprises a fatty acid, preferably, a-linolenic acid (omega-3), oleic acid (omega-9), linoleic acid (omega-6), or a combination thereof. In another embodiment, the extract further comprises a sterol, preferably, p-sitosterol, schottenol (i. e., stigmastenol), or a combination thereof.

In another embodiment, the extract further comprises a triterpene alcohol, such as a-amyrin, p-amyrin, 24-methylene parkeol, or a combination thereof. In still another embodiment, the extract further includes a phenolic compound, preferably, methoxyphenylpropionic acid, methoxycinnamic acid, or a combination thereof.

In another aspect, the invention provides a therapeutic composition (e. g. a foodstuff, dietary supplement, or pharmaceutical composition) comprising isolated cranberry seed oil or one or a combination of the above listed compounds derived from cranberry seed oil. Accordingly, the composition can contain one or more of the following components: a tocochromanol (e. g., a-tocopherol. y-tocopherol, 6-tocopherol, a-tocotrienol, y-tocotrienol, b-tocotrienol, or a combination thereof). an exogenous flavonoid (e. g., flavone, flavavone. isoflavone, or flavonol) a fatty acid (e. g.. a-linolenic acid (omega-3). oleic acid (omega-9), linoleic acid (omega-6), or a combination thereof) a sterol (e. g., p-sitosterol, schottenol (i. e., stigmastenol), or a combination thereof), a triterpene alcohol (e. g., a-amyrin, ss-amyrin, 24-methyleneparkeol, or a combination thereof), or a phenolic compound (e. g.. methoxyphenylpropionic acid, methoxycinnamic acid, or a combination thereof).

In another aspect, the invention provides a method for treating or preventing a disease or condition in a subject such as a malignancy, a hypercholesterolemic-related disease, a thrombotic disease, a respiratory disease, an atherogenic disease, an inflammatory disease or condition, a neurological disease. a dermatological disease, an opthalmological disease, or a gastroenterological disease, by administering to the subject a therapeutically-effective amount of a therapeutic composition (e. g., foodstuff, dietary supplement, or pharmaceutical composition) of the invention.

In a related embodiment, the subject has, or is at risk for acquiring, a malignancy, and is administered a composition comprising a tocotrienol, a flavonoid, tamoxifen, or a combination thereof.

In another related embodiment, the subject has or is at risk for acquiring a hypocholesterolemic-related disease, and is administered a composition comprising atocopherol. a-tocotrienol, y-tocotrienol, b-tocotrienol, or a combination thereof.

In yet another related embodiment, the subject has, or is at risk for acquiring, a respiratory disease, an inflammatory disease, a neurological disease, a dermatological disease, an opthalmological disease, or a gastroenterological disease and is administered a composition comprising a-tocopherol.

In another aspect, the invention provides a method for treating, preventing, or lowering the risk of acquiring a disorder or condition associated with an alteration in membrane stability, membrane fluidity, 5-lipoxygenase activity, or protein kinase C activity in a subject containing, the step of administering to the

subject a therapeuticallyeffective amount of a therapeutic composition (e. g., foodstuffs, dietary supplements, or pharmaceutical compositions) of the invention.

In another aspect, the invention provides a method for nutritionally supplementing a foodstuff by adding to the foodstuff an isolated extract, or one or more components derived therefrom. Accordingly, in one embodiment, the foodstuff, comprises a tocochromanol (e. g., a-tocopherol, y-tocopherol, 6-tocopherol, a-tocotrienol, y-tocotrienol, b-tocotrienol, or a combination thereof) an exogenous flavonoid (e. g., flavone, flavavone. isoflavone. or flavonol), tamoxifen, or a combination thereof, a fatty acid (e. g., a-linolenic acid (omega-3), oleic acid (omega-9), linoleic acid (omega-6), or a combination thereof). a sterol (e. g., p-sitosterol, schottenol (i. e., stigmastenol), or a combination thereof), a triterpene alcohol (e. g, a-amyrin, p-amyrin, 24-methylene parkeol, or a combination thereof), or a phenolic compound (e. g., methoxyphenylpropionic acid, methoxycinnamic acid, or a combination thereof).

In another aspect, the invention provides a method for isolating cranberry seed oil to a high level of purity, for example, so that the cranberry seed oil or components thereof can be administered to a patient or to an animal as a therapeutic. In one embodiment, the method involves physically disrupting cranberry seeds, adding to the seeds an organic solvent to produce an extract/solvent mixture, separating the extract/solvent mixture from the cranberry seeds, and removing the solvent portion of the extract/solvent mixture resulting in an isolated cranberry seed oil essentially free of solvent. In a preferred embodiment, the organic solvent is hexane and the adding step is conducted at a temperature between 50 and 90 F, and, more preferably, at a temperature between 50 and 65 F. In another embodiment, the separating step includes an extract receiver maintained at room temperature and atmospheric pressure. In another embodiment, the removing step is conducted at a temperature between 30 and 220 F and under vacuum, preferably, a vacuum pressure of 22 inches of Hg or greater.

In one embodiment, the extraction method results in an extraction yield by weight that is at least 10% or greater, preferably 15% or greater, and more preferably, at least 20% of the total weight of original seed or greater. In another embodiment, the extraction method also includes the step of increasing the oxidative stability of the resultant
extract, by exposing the extract to ascorbic acid, BHT, low temperature, or a combination of these conditions.

In a related aspect, the invention provides isolated cranberry seed oil produced by the above-mentioned extraction method. Accordingly, the cranberry seed oil is substantially free of impurities. In one embodiment, the isolated cranberry seed oil contains a tocochromanol (e. g., a a-tocopherol, y-tocopherol, 6-tocopherol, a- tocotrienol, y-tocotrienol, 6-tocotrienol, or a combination thereof. an exogenous flavonoid. an exogenous tamoxifen, a fatty acid (e. g., a-linolenic acid (omega-3), oleic acid (omega-9), linoleic acid (omega-6)), a sterol (e. g."ss-sitosterol schottenol (i. e., stigmastenol), a-amyrin, p-amyrin, 24-methylene parkeol), a phenolic compound (e. g., methoxyphenylpropionic acid, methoxycinnamic acid, or a combination thereof) or any combination of the above components. In a related embodiment, the isolated cranberry seed oil is contained in a foodstuff, dietary supplement, or pharmaceutical composition.

Accordingly, in another aspect, the invention provides a foodstuff dietary supplement, or pharmaceutical composition comprising isolated cranberry seed oil, or one or more components thereof, produced by a method of the invention.

Brief Description of t/e Drawings
Figure I shows a flow chart for extracting cranberry seed oil from cranberry seeds. The method shown is particularly suitable for small scale production of cranberry seed oil.

Figure 2 shows a detailed diagram of an apparatus for solvent extraction of cranberry seed oil from cranberry seeds.

Figure 3 shows a flow chart for extracting cranberry seed oil from cranberry seeds. The method shown is particularly suitable for large scale production of cranberry seed oil.

Figure 4 shows a schematic of a physical plant for large scale cranberry seed oil production using a Crown extractor.

Figure 5 shows a detailed diagram of a Crown extractor.

Figure 6 shows the chemical structure of two major sterols in cranberry seed oil, p-sitosterol and schottenol (i. e., stigmastenol).

Figure 7 shows the chemical structure of three major triterpene alcohols in cranberry seed oil, p-amyrin, a-amyrin, and 24-methylene parkeol.

Figure 8 shows the results of a high performance liquid chromatography (HPLC) analysis of two phenolic compounds in cranberry seed oil, methoxyphenylpropionic acid and methoxycinnamic acid.

Figure 9 shows a mass spectrum analysis of the phenolic compound methoxyphenylpropionic acid found in cranberry seed oil and the chemical structure of the compound methoxyphenylpropionic acid (insert panel).

Figure 10 shows a mass spectrum analysis of the phenolic compound methoxycinnamic acid found in cranberry seed oil and the chemical structure of the compound methoxycinnamic acid (insert panel).

Figure II shows the chemical structure of the a-, ss-, y-, 6-tocotrienol (top panel) and the chemical structure of a-, ss-, Y-tocopherol (bottom panel).

Detailed Description of t/re Invention

The invention provides an isolated and highly pure cranberry seed oil extract, a novel source of several therapeutically beneficial compounds, which can be administered to animals or humans, for example, in the form of a foodstuff, dietary supplement, or pharmaceutical composition. Accordingly, in particular embodiments, the invention features a foodstuff, dietary supplement, or pharmaceutical composition comprising isolated cranberry seed oil or one or a combination of components derived therefrom. The invention also provides methods for efficiently extracting cranberry seed oil from cranberry seeds to a high level of purity.

These and other elements of the invention are described below.

Definitions

As used herein, the term"isolated"refers to cranberry seed oil isolated from its natural context (i. e., cranberry seeds)."Isolated cranberry seed oil"and"cranberry seed oil extract"are used interchangeably herein. Preferably,"isolated cranberry seed oil"of the invention has high stability against oxidation, good light-golden color, long shelf life, resistance to gelling, a pleasant flavor, a high tocochromanol content (especially tocotrienols having anti-carcinogenic properties), rich in omega-3-acid alpha linolenic acid, and is substantially free of free fatty acids (especially, saturated fatty acids, e. g., palmitic acid, known to be a major contributor to heart disease) and impurities.

The terms"substantially free of impurities"and"high level of purity"refers to cranberry seed oil which is substantially (e. g., at least 80-90%, preferably 90-99%. more preferably greater than 99%, and most preferably greater than 99.7%) free of solvent as determined by smell and gas chromatography, free of peroxides (as determined by a very low peroxide value, e. g., less than 7meq/Kg), free of free fatty acids (as determined by titration, e. g., less than 0.8% oleic), free of solids, e. g., hull and other particles (e. g., as determined by a crystal clear appearance), and/or off-flavors (as determined by taste and smell).

The term"tocochromanol"refers to any tocopherol (T) or tocotrienol (T3) compound, for example, a-tocopherol, y-tocopherol, b-tocopherol, a-tocotrienol, y- tocotrienol, 6-tocotrienol, or a combination thereof, that is present in measurable levels in cranberry seeds.

The term"native"means the component is derived from cranberry seed.

The term"exogenous"means the component is derived or obtained from a source other than cranberry seeds. Exogenous compounds suitable for adding to a cranberry seed oil extract include, for example, tamoxifen, and various flavonoids (e. g., a flavone, a flavavone, a isoflavone. or a flavonol).

The term"fatty acid"refers to a fatty acid that is naturally present at some measurable level in cranberry seeds and includes, for example, a-linolenic acid (omega3), oleic acid (omega-9), linoleic acid (omega-6),

or a combination thereof.

The term"sterol"refers to any sterol (e. g., that is naturally present at some measurable level in cranberry seeds (there are at least 60 of which 14 have been characterized)) and includes, for example, p-sitosterol and schottenol (i. e., stigmastenol).

The term"triterpene alcohol"refers to a triterpene alcohol (e. g., that is naturally present at some measurable level in cranberry seeds (there are at least 22, of which 7 have been identified)), and includes, for example, a-amyrin, p-amyrin, and 24methylene parkeol.

The term"phenolic compound""refers to a phenolic compound (e. g., that is naturally present at some measurable level in cranberry seeds) and includes, for example, methoxyphenylpropionic acid and methoxycinnamic acid.

The term"foodstuff'refers to any edible substance that can be used as or in food for an animal or human. Foodstuffs include substances that may be used in the preparation of foods such as cooking oils or food additives. Foodstuffs also include animals or animal products used for human consumption, such as. for example eggs or milk. Such animal themselves can be fed or treated with a composition of the invention and retain the advantageous properties of the composition (e. g., isolated cranberry seed oil or components thereof) or impart those advantageous properties to products such as eggs or milk.

The term"dietary supplement"refers to a compound or composition used to supplement the diet of an animal or human.

The term"pharmaceutical composition"refers to a composition formulated for therapeutic use.

The term"major components"refers to a component generally found in the extracts of the invention in amounts greater than 1% by weight (e. g., a fatty acid).

The term"minor components"or"trace components"refers to a component generally found in the extracts of the invention in amounts less than 1 % by weight (e. g., sterols, triterpene alcohols, phenolic compounds, and tocochromanols).

Cranberry Seed Oil (CSO) Extract And Its Components
Cranberry seed oil (CSO) extracts of the invention provide a novel source of several therapeutically beneficial compounds, such as omega-3, omega-6, and omega-9 fatty acids, tocochromanols and sterols. CSO extracts of the invention also provide several advantages over currently known sources of such therapeutically beneficial compounds including, for example, a remarkably high concentration of particularly desirable components (e. g., omega-3 fatty acids, tocochromanols and sterols), low levels of undesirable fatty acids (e. g., palmitic oil), and a high level of purity, for example, so that the CSO or components thereof can be used in foodstuffs, or as dietary supplement or pharmaceutical composition.

Accordingly, in one embodiment, the invention provides a CSO extract, or a composition comprising one or more major and/or minor components of a CSO extract, as listed in Table 1, which promotes health in a human or other animal. The CSO extract or composition derived therefrom is also preferably substantially free of impurities and low in undesirable fatty acids. The CSO extract or composition derived therefrom also can contain one or more exogenous (i. e.. externally added) compounds to further enhance the therapeutic value of the CSO extract or composition derived therefrom, for example, by acting in synergism with one or more native components of the CSO extract.

The terms"health promoting"',"therapeutic"and"therapeutically active"are used interchangeably herein, and refer to the prevention or treatment of a disease or condition in a human or other animal, or to the maintenance of good health in a human or other animal, resulting from the administration of a CSO extract of the invention, or a composition derived therefrom. Such health benefits can include, for example, nutritional, physiological, mental and neurological health benefits.

As shown in Table 1 (below). CSO extracts of the invention can contain one or more of the following compounds: fatty acids, e. g., a-linolenic acid (omega-3), oleic acid (omega-9), and linoleic acid (omega-6); sterols, e. g.,-sitosterol or schottenol (i. e., stigmastenol); triterpene alcohols, e. g., a-amyrin,-amyrin, or 24-methylene parkeol; phenolic compounds, e. g., methoxyphenylpropionic acid, and methoxycinnamic acid;

tocochromanols, e. g., a-tocopherol, y-tocopherol, 6-tocopherol, a-tocotrienol, ytocotrienol, and 6-tocotrienol. In addition, exogenous compounds, such as flavonoids and/or tamoxifens, can be added to CSO extracts of the invention and compositions derived therefrom, to achieve a synergistic effect.

Table 1. Major and Minor Components of Cranberry Seed Oil

MAJOR COMPONENTS MINOR (TRACE) COMPONENTS
1. Omega-3 Fatty Acids 1. Sterols, 60 detected, 14 identified, 2 characterized
a-linolenic acid (33%) (0. 12%)
B-sitosterol (delta-5)
2. Omega-6 Fatty Acids Schottenol (stigmastenol) (delta-7)
Linoleic acid (38%) 2. Triterpene Alcools, 22 detected, 7 identified, 3 characterized (0.6%)
3. Omega-9 Fatty Acids a-amyrin
Oleic acid (21%) ss-amyrin
24-methylene parkeol
3. Phenolic Compounds, 2 characterized
methoxyphenylpropionic acid
methoxycinnamic acid
4. Tocopherols
a-tocopherol-130 ppm
P-tocopherof-trace
y-tocopherol 110 ppm
S-tocopherol 16 ppm
5. Tocotrienols
a-tocotrienol-180 ppm
/3-tocotrienol 0 ppm
y-tocotrienol 1,500 ppm
S-tocotrienol 50 ppm
* Percentages (%) are by weight * ppm = parts per million I. MAJOR COMPONENTS
One class of major components found in cranberry seed oil extracts of the invention are the fatty acids. In particular, cranberry seed oil extracts of the invention are rich in omega-3, omega-6, and omega-9 fatty acids. Typically, the cranberry seed oil extract contains, by weight, approximately 30-38%, (typically about 33%) alinolenic acid (omega-3), 35-39% (typically about 38%) linoleic acid (omega-6), and 2022% (typically about 21 %) oleic acid (omega-9). Moreover, cranberry seed oil extracts of the invention have the additional advantages of being edible, having a pleasant flavor, and preferably having good oxidative stability.

In contrast. other known sources of fatty acids lack these advantages. For example, flaxseed oil (linseed oil) is not an edible oil, but rather a"drying"oil used in the painting industry. Oils from soybean, fish, rapeseed, and canola lack the pleasant flavor and the presence of beneficial tocotrienols. In addition, fish oil lacks the stability against oxidation exhibited by cranberry seed oil. Moreover, none of these oils have the superior combination of therapeutic compounds found in cranberry seed oil.

Specifically, while these oils have omega-3 fatty acids. isolated cranberry seed oil of the invention also has both omega-6 and omega-9 fatty acids which play important roles in various health aspects.

Fatty acids
The omega-3 fatty acids contained in the cranberry seed oil extracts of the invention are essential for growth and development throughout the life cycle. For example, omega-3 fatty acids are known to play an important role in, 1) the normal function of the retina and brain, especially in new born infants, 2) maintaining favorable serum triglycerides in normal subjects and in patients with hypertriglyceridemia, 3) the normal function of the vascular and neurological systems, and 4) reducing LDL (low density lipoprotein) cholesterol in patients with hyperlipidemia (provided that the saturated fatty acid content in the diet is decreased).

In normolipidemic subjects, omega-3 fatty acids can prevent and rapidly reverse the carbohydrate-induced hypertriglyceridemia, decrease platelet aggregation, lower blood viscosity, decrease fibrinogen levels,

lower tendency for thrombus formation, inhibit production of platelet-derived growth factor (PDGF), increase endotheliumderived relaxing factor (EDRF), and inhibit production of platelet activating factor (PAF). Moreover, the omega-3 fatty acids can also function as an anti-inflammatory and reduce joint pain in patients with rheumatoid arthritis. Still further, omega-3 fatty acids have been linked to a role in gene expression, benefiting patients with ulcerative colitis, decreasing the toxicity of cyclosporin in patents with psoriasis, and improving skin lesions.

Similarly, the omega-6 (linoleic acid) and omega-9 (oleic acid) fatty acids, also derivable from cranberry seed oil extracts of the invention, play important roles in normal physiological functions. In addition. these fatty acids have also been associated with various health benefits relating to overall growth, healthy skin. reproduction, and cardiovascular health.

Accordingly, formulations can be prepared by those of ordinary skill in the art containing one or a combination of the above-mentioned desirable fatty acids. Such formulations have application in the medical and pharmaceutical industries for enhancing, maintaining or treating any of the above-mentioned biological functions or disfunctions. In addition, given the wide spectrum of biologic processes affected by these fatty acids, the cranberry seed oil extract of the invention can also be used as a food additive or dietary supplement.

For example, in the food industry, to raise the availability of desirable fatty acids in a consumer's diet, the cranberry seed oil extract of the invention, or compositions derived therefrom (e. g., containing components or fractions thereof), can be added to, for example, juices, bakery products, infant formulas, etc. As dietary supplements, the cranberry seed oil extract of the invention or compositions derived therefrom can be taken in the form of e. g, liquids, pills, or capsules as are known in the art. As discussed further below, methods for formulating such vehicles of administration can be performed using standard techniques.

In another embodiment, the cranberry seed oil extract of the invention or compositions derived therefrom (e. g., containing health-promoting fatty acids) can be fed or otherwise administered to laying hens to produce eggs rich in desirable fatty acids, or to cows or other livestock to produce meat and dairy products rich in such fatty acids. The resultant food products derived from these animals can then be consumed by humans for their enhanced nutritional and health benefits.

Alternatively, the cranberry seed oil extract of the invention or compositions derived therefrom can be fed or otherwise administered to animals, such as pets or domesticated livestock, for therapeutic purposes (e. g., to correct problems such as dry skin, allergic reactions, and cancer).

II. MINOR COMPONENTS

Cranberry seed oil extracts of the invention also contain a number of minor components having significant therapeutic value.

Sterols

In particular, cranberry seed oil extracts of the invention can contain one or more sterols. including, but not limited to.-sitosterol (A-5) and schottenol (A-7) (also referred to as stigmastenol).

Plant sterols (phytosterols) have been shown to inhibit the absorption of cholesterol absorption from the intestine, and decrease blood serum cholesterol. It has been proposed that, in the intestine, phytosterols act by reducing the solubility of cholesterol in the lipid and micellar phases with a consequential decrease in cholesterol absorption. Plant sterols are also reported to inhibit colon cancer development.

Accordingly, the cranberry seed oil extracts of the invention and compositions derived therefrom (e. g., fractions rich in phytosterols) can be used, for example, in the treatment of patients with hypercholesterolemia or as chemopreventative agents against colon cancer.

Triterpene Alcohols

In addition, cranberry seed oil extracts of the invention also can contain one or more triterpene alcohols. As part of the present invention, several triterpene alcohols were identified including, but not limited to,-amyrin, a-amyrin, and 24-methylene parkeol, the three primary alcohols. Such triterpene alcohols are known to confer significant health benefits, e. g., against heart disease and cancer, due to their strong antioxidant properties.

Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom (e. g, fractions rich in triterpene alcohols) can be used to treat diseases including, but not limited to heart disease and cancer.

Pl1enolic Compounds
Cranberry seed oil extracts of the invention also can contain one or more (e. g., at least two) phenolic compounds, such as methoxyphenylpropionic acid and methoxycinnamic acid.

Such phenolic compounds can act as potent antioxidants and, therefore, can prevent or delay oxidation rections which cause various diseases. Accordingly, the cranberry seed oil extracts of the invention and compositions derived therefrom can be used as used as anti-oxidants. For example, they can inhibit lipid peroxidation, scavenge free radicals and active oxygen, inactivate lipoxygenase, and chelate iron ions.

They also can be used to inhibit erythrocyte aggregation and sedimentation. Moreover, epidemiological studies have demonstrated that the consumption of phenolic compounds is associated with a reduced risk of cancer. Accordingly, the cranberry seed oil extract of the invention and compositions derived therefrom (e. g., fractions rich in phenolic compounds) can be used to treat cancer with fewer side effects compared to standard chemotherapies.

Tocochromanols (Tocopherols and Tocotrienols)
Cranberry seed oil extracts of the invention also contain a remarkably high concentration of tocochromanols (a class of compounds that includes tocopherols and tocotrienols). such as a-tocopherol, y-tocopherol, b-tocopherol, a-tocotrienol, ytocotrienol. 6-tocotrienol, or a combination thereof. A large body of research has shown the importance of tocopherols and tocotrienols in the defense against numerous biological disorders. To date, palm oil is the only other edible oil known to contain tocotrienols in a significant amount, however, isolated cranberry seed oil of the invention has two major advantages over palm oil. First, cranberry seed oil extracts of the invention contain a much higher concentration of the beneficial y-tocotrienol (about
1,600 mg/kg) as compared to palm oil (400 mg/kg). Second, cranberry seed oil extracts of the invention have the superior advantage over palm oil of having very little of the undesirable palmitic acid (only 6% vs. 46%), the saturated fatty acid thought to contribute to heart disease.

Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom (e. g., fractions rich in tocochromanols) can be used to treat respiratory, inflammatory, neurological, dermatological. opthalmological. and gastroenterological diseases. Surprisingly. the amount of tocotrienols determined to be in the cranberry seed oil extract of the invention (a total of more than 1,700 mg/Kg) exceeds that in any other oil known so far. In particular, cranberry seed oil is remarkably rich (~ 1.500 mg/Kg) in y-tocotrienol which has been shown to be, in most cases, the more biologically active of the tocotrienol isomers. In contrast, palm oil has a total tocotrienol content only of approximately 500-700 mg/Kg. with far less of the desirable y-T3 (only 280-400 mg/Kg compared to cranberry seed oil which has approximately 1,500 mg/Kg). Other food oils, for example, barley, rice, and rice bran oils and brewers grain. contain in the range of 400-700 mg/Kg y-T3. However, their importance as edible oils is negligible in view of the very small amounts that can be economically extracted from the grains. In addition, several of these grains contain oil susceptible to enzymatic hydrolysis, e. g., rice bran oil. All other known edible oils are extremely poor in y-T3. Finally, the cranberry seed oil extracts of the invention also have a pleasing flavor and aroma.

Tocochromanols-Stnucture. Nomenclatzrre, and Prevalence
The tocochromanols include the major active components of vitamin E and are capable of alleviating vitamin E deficiency symptoms. The tocochromanols include the tocopherols (T) and the tocotrienols (T3). All have derivatives which differ according to the position and number of methyl groups present on the chromanol ring and are designated as the a, ss*, and 6-isomers. The side chains consist of three isoprenoid units which, in the case of the tocopherols (Fig. 11, bottom), are completely saturated while the tocotrienols have double bonds at positions 3', 7', and 11' (Fig. 11, top).

Distribution and Sources of Tocotrienols
In general, tocopherols predominate in oil seeds and green parts of higher plants, whereas the tocotrienols predominate in the aleurone and subaleurone layers of cereal seeds (especially rice bran oil and barley oil) and in palm oil. The distribution of tocopherols and tocotrienols in some common oils (Table 2) and their distribution in cereals and brans (Table 3) is provided below. Prior to the instant invention. palm oil was the

most practical source of tocotrienols.

Table 2. Approximate Content of Tocopherol and Tocotrienol Found in Vegetable Oils (mg/kg)

Tocopherols Tocotrienols
γ-T#-Tα-T3ss-T3γ-T3@α-Tss-T
-555Trace1-20Coconut5-10
Cottonseed 40-560 270-410 0---
Maize,grain 60-260 0 400-900 1-50-0 0-240 0
Maize, 1-20450-7905-60----300-430
Olive 1-240 0 0 0---
Trace32070120-15020-40260-30070Palm180-260
Peanut 80-330-130-590 10-20---
-280-59010-20----Rapeseed/canola180-280
-70-190230-240----Safflower340-350
Soybean 30-120 0-20 250-930 50-450 0 0 0
Sunflower 350-700-10-50 1-10--
20-40590450----Walnut560
Wheat660-26027020-9080-190--560-
1200 810

Table 3. Tocopherols and Tocotrienols in Different Cereals and Brans

Cereals and Tocopherols (ppm) Tocotrienols (ppm)
their brans a-T-T y-T 6,-T a-T3-T3 y-T3 6,-T3
Wheat 14 7 0 0 33 0 0 0
Wheatgerm 239 90 0 0 30 100 0 0
Wheatbran 16 10 0 0 13 55 0 0
Corn 6 0 45 0 3 0 5 0
Oat 5 1 0 0 11 2 0 0
Rye 16 4 0 0 15 8 0 0
Rice,white 1 0 1 0 1 0 2 0
Rice, brown 6 1 1 0 4 0 10 0
Rice, bran 6 1 0 8 1 4 4 6
Rice bran 3 15 4 2 1 14 22 29
Barley 2 4 0 1 11 3 2 0
Barley bran 11 16 36 4 36 25 19 11
Brewers grain 31 42 114 20 199 40 39 34
Diet, Vitamin E, and Cancer
It has been shown that increasing fat-derived energy in the diet, and increasing the linoleic acid content of diets at constant fat-derived energy, results in increased tumorigenicity (Birt et al., J. Clin. Nutr., 45: 203-209 (1987), Birt et al., Nutr. Rev., 48: 15 (1990); Carroll et al., Current Opinion in Lipidology, 8: 53-56 (1997); Erickson et al.,
Nutr. Rev., 48: 6-14 (1990); Ip et al., Cancer Res., 45: 1997-2001 (1985); Ip et al., Am. J.

Clin. Nutr., 45: 218-224 (1987); Thompson et al., Cancer Res., 49: 1904-1908 (1989);
Welsch et al., Am. J. Clin. Nutr., 45: 192-303 (1987)). The effect of linoleic acid is thought to occur via its influence on prostanoid metabolism, immune response, or cell membrane structure and function. Increasing the fat-derived energy content of diets of equal linoleic acid content by the addition of palm oil did not enhance tumorigenesis in moderately exercised rats (Thompson et al., Cancer Res., 49: 1904-1908 (1989)).

Results reported by Sundram, et al. (Cancer Res., 49: 1447-1451, (1989)) suggest that crude palm oil is more effective than refined, bleached, and deodorized palm oil in increasing the latency (the interval between administration of a carcinogen and appearance of a palpable tumor) of 7,12-dimethybenz (a) anthracene (DMBA)-initiated tumorigenesis.

The use of vitamin E as an anticarcinogenic agent has been recognized for a number of years (Haenszel et al., Int. J. Cancer, 36: 43-48 (1985); Menkes et al., N.

Engl. J. Med., 315: 1250-1204 (1986); Stahelin et al., Ann. NYAcad Sci., 570: 391-399 (1989)). In addition, in vitro and in vivo studies, including human studies. have demonstrated that vitamin E interferes with the development of carcinogenesis that results from exposure to various environmental factors known to enhance oxidant stress (Borek et al., In, Mechanisms of cellular transformation by carcinogenic agents, New
York, Pergamon (1987), Borek et al., In, Medical, biochemical and chemical aspects of free radicals, Amsterdam, Elsevier, (1989); Borek et al., Proc. Natl. Acad. Sci. USA 83: 1490-1494 (1986); Proc. Natl. Acad. Sci. USA, 88: 1953-1957 (1991)). In addition, a-tocopherol, a component of vitamin E, is a hydrophobic, peroxyl radical trapping, chain-breaking antioxidant found in biological membranes. Accordingly, the protective role vitamin E plays in inhibiting a variety of human malignancies is mainly attributed to its components having the ability to protect the lipid material of the organs against oxidation (Ames et al., Science 230: 271-279 (1987); Doll et al., J. Natl. Cancer Inst.

66: 1193-1194 (1981): Greenwald et al., Cancer 65: 1483-1490 (1990); Menzel et al., J.

Agr. Food Chem., 20: 481-486 (1972)).

Metizodsfor Evaluating Titerapeutic Properties of Cranberry Seed Oil Extract
And Components Derived Tlterefrom
In Vivo Animal and Clinical Studies
In one embodiment, cranberry seed oil extracts of the invention and compositions derived therefrom can be tested for their therapeutic effect by administering (e. g., orally or by injection) the extracts or compositions in a suitable form (e. g., as a pharmaceutical composition or dietary supplement) to a human or other animal, and then observing the physiological effect (e. g., compared to a control). The human or animal can, for example, be suffering from a disease or condition, such as those described herein (e. g., cancer, hypercholesterolemia or heart disease). Thus, a reduction in the physical symptoms of the disease can be measured as an indication of the therapeutic efficacy of the cranberry seed oil extract or compositions derived therefrom.

Cell Proliferation Assays
The health promoting properties of cranberry seed oil extracts of the invention and compositions derived therefrom also can be evaluated using a variety of artrecognized cell proliferation assays. Suitable methods include, for example, those described below.

To evaluate anti-tumor activity, cranberry seed oil extracts of the invention or compositions derived therefrom (e. g., a fraction thereof) can be used in a controlled animal study. In general, tumors are induced in the animal via diet, by applying chemical tumor promoter to the skin, or by the implantation of tumor cells in the presence or absence of the test agent. Various assays, such as those described below, can then be used to examine the progress of carcinogenesis in the presence or absence of the administration of the extracts or compositions of the invention.

In one embodiment, a tumor cell proliferation assay is performed by measuring the incorporation of [3H] thymidine into the DNA of dividing cells, as is known in the art. For example, a solution containing a cranberry seed oil extract of the invention or components derived therefrom (e. g., a tocopherol or tocotrienol rich fraction) can be added to tissue culture plates, for example, in decreasing concentrations and incubated at 37 C for 3 days, after which tritiated thymidine is added to each well to determine the number of dividing cells at each concentration. The cells are further incubated for a sufficient period of time, e. g, 4 hrs, to allow for the incorporation of the radiolabel into the DNA of dividing cells and then medium and excess label are removed. The cells can then be harvested by, e. g., trypsinization, and the amount of radioactivity present in the cells is measured using standard techniques. The concentration at which the extracts of the invention exhibit 50% inhibition of cell growth (IC50) is determined by comparing the radioactivity measured in the extract-treated cells as compared to the untreated control cells.

ViabilityAssays
To assess the viability of tumor cells after exposure to a cranberry seed oil extract of the invention or a composition derived therefrom, the cells can be mixed with 3- [4-5-dimethylthiazol-2-yl)-2,5-diphenyl-

tetrazolium bromide (MTT). The intensity of the blue color, due to a formazan product formed by cellular reduction of MTT by the mitochondrial dehydrogenase of the surviving cells, is then measured as an indication of the viability of the remaining cells (Hansen et al., J. Immunol. Methods, 119: 203-210 (1989)). Percent viability can be determined by relating absorbance/concentration of the treated cells to that of the non-treated controls.

Long term Gro^th Sssays
The long term growth effects on cells caused by exposure to a cranberry seed oil extract of the invention or a composition derived therefrom can be determined by incubating plates containing the cell culture medium plus the reagent at its IC50 concentration at 37 C. Plates are removed at appropriate intervals, the medium aspirated, the cells trypsinized, resuspended, counted with a hemocytometer, and the number of cells plotted against time to construct growth curves.

MetAlods of Use
Treatment of Cancer
In one embodiment, a cranberry seed oil extract of the invention and compositions derived therefrom (particularly those having high tocotrienol content) can be administered to a human or other animal to treat or prevent a variety of cancers. The extract and compositions derived therefrom also can be administered in combination with other anti-cancer agents. In particular, the cranberry seed oil extracts of the invention and compositions derived therefrom can be administered with either tamoxifen and/or a flavonoid for the treatment of, for example, breast cancer. These combinations of agents encompassed by the invention are particularly effective because of the ability of tocotrienols to act in synergy with tamoxifen and/or flavonoids in the inhibition of tumorigenic cells.

For example. it is known that most breast cancers consist of hormone-dependent as well as hormone independent cells. The drug tamoxifen. a synthetic non-steroida) estroten antagonist, has been widely used in the treatment of hormone-responsive breast cancer. In addition. the inhibitory effects of various combinations of the palm oil tocotrienol-rich fractions as well as individual tocotrienols in combination with tamoxifen on at least two breast cancer cell lines (i.e., estrogen receptor-negative MDAestrogenreceptor-positiveMCF-7)havebeendemonstrated(seeTable4)MB-435and (Guthrie. et PacificJ.Clin.Nutr.41-45 (1997)).Asia
Table 4. Inhibition of Proliferation of MDA-MB-435 and MCF-7 by Combinations of Tocotrienols with Tamoxifen

MDA-MB-435 MCF-7
IC50, g/mLInhibitorIC50, g/mL
α-Tocopherol 125#3
TRF 180+3 4+0.1
α-Tocotrienol 6#0.3
γ -Tocotrienol 2#0.1
5-Tocotrienol 90 + 3 2 + 0.05
Tamoxifen90+40. 04+0.001
a-Tocopherol + Tamoxifen 46. 9 + 2
TRF + Tamoxifen 3.9 # 0.2 0.5 # 0. 02
α-Tocotrienol + 0.1#0.0051.5#0.05
y-Tocotrienol + Tamoxifen 1.9#0.02 0. 01 + 0.0002
8-Tocotrienol + Tamoxifen 5.9#0.1 0. 003 + 0.0001

In particular. it was concluded with regard to MDA-MB-435 cells. that gammatocotrienol was a much more effective inhibitor of proliferation than tamoxifen alone.

However, when TRF. a-tocopherol, a-, y-or b-tocotrienols were combined in equimolar concentration with tamoxifen. the combinations inhibited cell proliferative much more effectively than when used alone. Importantly. these studies showed that most cells were viable at the IC50 concentration at which compounds were added suggesting that the anti-tumor compounds are not toxic when administered at therapeutically effective dosages. A synergistic effect was also evident when these combinations of compounds were tested on cell growth over a longer period of time.

With regard to studies performed using MCF-7 breast cancer cells, tocotrienolrich fractions were shown to inhibit the proliferation of MCF-7 cells more effectively than a-tocopherol, but not as effectively as tamoxifen. In addition, the tocotrienols gave much lower ICso in MCF-7 cells than in MDA-MB-435 cells with y-T3 and 6-T3 being the most effective. In most cases, however, the compounds used in combination with tamoxifen (1: 1) showed ICso values intermediate between those of the individual compounds used alone. Only y-T3 and 6-T3 gave lower ICso values when combined with tamoxifen than when used alone, with the 6-T3/tamoxifen combination being remarkably potent. Similar inhibitory effects of these combinations were observed on cell viability and growth.

Prior to the present invention, treatment of cancer patients with tamoxifen had several drawbacks. For example, tumors can develop resistance to tamoxifen, possibly caused by the drug's intrinsic estrogen antagonist properties (Osborne et al., J. Natl.

Cancer Inst. 87: 746-750 (1995)). Also, tamoxifen may increase the incidence of new primary malignancies, e. g. endometrial, liver, and colorectal cancers (Rutgrist et al., 1995). Accordingly, the present invention provides the advantage of enabling the administration of tamoxifen in lower doses, for example, in combination with a cranberry seed oil extract of the invention or a composition derived therefrom (particularly one having a high tocotrienol content) to avoid these undesirable effects.

Synergy with Flavonoids
In another embodiment, a cranberry seed oil extract of the invention or a composition derived therefrom is administered in combination with tamoxifen as described above and/or in combination with a flavonoid for the treatment or prevention of cancer. These combinations of agents encompassed by the invention are particularly effective because of their known ability to act in synergy, as demonstrated below, in the inhibition of tumorigenic cells.

Flavonoids are polyphenolic compounds which occur in plant foods, particularly citrus. These compounds include the flavones, e. g. tangeretin; the flavavones, e. g. hesperetin; the isoflavones, e. g. genistein; and the flavonols, e. g. quercetin. Several studies have demonstrated the anticancer properties of flavonoids from various plant sources (Cook et al., J. Nutr. Biochem. 7: 66-76 (1996); Hertog et al., Nutr. Cancer 20: 21-29 (1993); Middeleton et al., Trends Pharm. Sci., 5: 335-338 (1984)). Further, various combinations of flavonoids from different sources have been shown to be synergistic in their ability to inhibit the proliferation of a breast cancer cell line (MDAMB-435 cells).

In particular, synergistic effects between the tocotrienols and flavonoids, with y-
T3 and tangeretin being the most effective combination, have been observed when tested for their ability to inhibit growth in MDA-MB-435 and MCF-7 breast cancer cells (IC50 0.05 pg/mL and 0.02 g/mL, respectively) (Guthrie et al., Asia Pacific J. Clin. Nutr.

6: 41-45 (1997)). In addition, with few exceptions, combinations (1: 1: 1) of tocotrienols, flavonoids. and tamoxifen were more effective than 1: 1 combinations of T3 and flavonoids, T3 and tamoxifen, or flavonoids and tamoxifen and these are summarized in
Table 5, below. The most potent combinations were γ-T3/tangeretin/tamoxifen with the MDA-MB-435 cells (ICso 0.01 ug/mL), and b-T3/hesperetin/tamoxifen (ICso 0.0005 i g/mL) with the MCF-7 cells.

Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocotrienol content) can be used in combination with tamoxifen and/or flavonoids as potent anti-cancer agents.
Table 5. Synergy of Tocotrienols with Tamoxifen and Flavonoids in the Inhibition of
Proliferation

MDA-MB-435 MCF-7
MCF
302γ-T3only
Tangeretin only 0.5 0.4
Tamoxifen only 90 0.04
y-T3 + tangeretin 0.5 0.02
y-T3 + tamoxifen 2 0.01.

Tangeretin + tamoxifen 0.5 0.08
γ-T3 + tangeretin + 0.01 0.02
tamoxifen
-T3 +

Moreover. a study involving 47 hypercholesterolemic subjects administered dietary supplements containing 200 mg of TRF per day for 4 weeks resulted in, respectively, a 15-22% and 10-20% reduction in serum total and LDL cholesterol (Quereshi, et al., Lipids 20: 817-824 (1985); Quereshi, et al., Am. J. Clin. Nutr., 53: 1021S-1026S (1991)). In addition, in studies where the hypocholesterolemic effect of tocotrienols was compared with that of other drugs, the tocotrienols were more effective. For example, in a study involving chickens, T3 was demonstrated as being twice as effective as LovastatinT5, a drug currently used for cholesterol control in humans. And most of the drugs most commonly used today in the therapy of hypercholesterolemia (i. e., Nicotinic acid (Grundy et al., J. Lipides., 22: 24-36 (1981)), CompactinTM and LovastatinTM (Illingworth et al., Eur. Heart J., Supp. E: 103-111 (1987) ; Endo et al. Biotechnology, 26: 301-320 (1994)), CholestyramineTM and ColestipolTM (Shepherd et al., Biochem. Soc. Trans. 15: 199-201 (1980)), Clofibrate and GemfibrosilTM (Kesaniemi et al., JAMA, 251: 2241-2246 (1984)) and Probucol are known to produce various side effects (Illingworth et al., Am. J. Cardiol., 60: 33G-42G (1987)). In contrast, no toxic effects were observed in the studies where tocotrienols were administered.

Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocotrienol content) can be used in the treatment of heart disease.

Tocotrienols in the Treatment of Other Diseases and Disorders
In yet another embodiment, cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocotrienol content) can be used in the treatment or prevention of a wide range of other diseases and disorders that include aging, respiratory, inflammatory, neurological, dermatological, opthalmological, and gastroenterological diseases. Indeed, a large volume of reported research provides evidence that vitamin E-containing tocochromanols plays a critical role in the above-mentioned conditions.

In addition, as presented herein, the tocotrienols, which are also members of the vitamin E family, have proved in many cases to be even more protective than a-tocopherol. Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom having both of these active compounds (i. e., tocopherols and tocotrienols) are especially well suited for the treatment of a broad spectrum of biological conditions linked to the action of tocopherols and/or tocotrienols.

Moreover, such extracts and compositions of the invention also are well suited to the treatment of any yet to be characterized biological disorders or diseases that, at some level, are affected by or controlled by a mechanism linked to the action of, a tocopherol or tocotrienol.

For example, cranberry seed oil extracts of the invention and compositions derived therefrom can be used to prevent endothelial injury, such as ischemic and reperfused myocardium and ulcers. In addition, the extracts and compositions can be used to inhibit tumor necrosis factor biosynthesis which, in turn, decreases inflammation (e. g., by inhibiting respiratory bursts of neutrophils or via free radical scavenging). Accordingly, cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocopherol and/or tocotrienol content) can be used as antiinflammatory agents for the prevention and treatment of a wide variety of diseases and conditions involving minor, acute and chronic inflammation.

These include, but are not limited to, fever, rheumatoid diseases, pain, functio laesa, hypertension and edema.

Cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocochromanol (e. g., tocotrienol) content) also can be used to treat glucose intolerance in diabetes mellitus, and/or to restore acute glucoseinduced insulin response in non-insulin-dependent diabetes mellitus. In addition to their role in inflammatory response, prostaglandins have also been shown to inhibit glucoseinduced insulin release, increase glucose concentration and stimulate glucagon secretion.

Consequently, use of the compounds of the invention can lead to an increased insulin to glucagon ratio.

In addition to the above-stated uses, cranberry seed oil extracts of the invention and compositions derived therefrom (particularly those having high tocotrienol content) can be used to enhance the immune response in animals and humans, for example, by reducing the amount of fatty acids in biological tissues. Since fatty acid levels effect the immune system, the compounds of this invention may serve as immunoregulators. They may, for example, be used to increase antibody titers to foreign proteins.

In addition, the reduction in fatty acid, cholesterol, fatty acid and/or glucose levels induced by the compounds of the invention can be obtained without attendant substantial weight loss, resulting in an increased feed to protein conversion ratio. Therefore, the extracts and compositions of the invention can be used to increase feed conversion efficiency.

Hypercholesterolemic diseases and conditions that can be treated using the cranberry seed oil extracts of the invention and compositions derived therefrom include, but are not limited to, atherosclerosis, arteriosclerosis, xanthomatosis, hyperlipoproteinemias, and familial hypercholesterolemia.

Thrombotic diseases and conditions that may be treated using cranberry seed oil extracts of the invention and compositions derived therefrom include, but are not limited to, pulmonary disease (for example, involving reduced conductance, compliance, or constriction), excessive fluid accumulation or pulmonary edema, respiratory distress, asthma, pulmonary vascular permeability, pulmonary vasoconstriction, pulmonary hypertension, pulmonary embolism, cardiac ischemia, myocardial infarction, cardiopulmonary bypass associated dysfunction, vasoconstriction, organ dysfunction, platelet dysfunction. cardiac disease, chronic obstructive arterial disease caused by arteriosclerosis, vasoconstriction, renal artery stenosis, myocardial infarction, stroke, deep vein thrombosis, peripheral arterial occlusion, and other blood system thromboses.

The antioxidizing properties of the cranberry seed oil extracts of the invention and compositions derived therefrom may also be applied to, but are not limited to, the treating and preventing of cancerous conditions by, for example, preventing or limiting cancer-causing mutations in the genetic material of an animal or a human.

Antiatherogenic diseases and conditions that can be treated using cranberry seed oil extracts of the invention and compositions derived therefrom include, but are not limited to, atherosclerosis, arteriosclerosis, myocardial infarction, ischemia (i. e., myocardial ischemia, brain ischemia, and renal ischemia) and strokes.

Inflammatory diseases and conditions that can be treated using cranberry seed oil extracts of the invention and compositions derived therefrom include, but are not limited to, essential hypertension, hypertension of congestive heart failure, renal dysfunction caused by reduced myocardia output, endotoxemia, chronic liver disease or hypertension, pulmonary inflammation in asthma, lung injury (bronchitis, pneumonia, or acute); rheumatic diseases (for example, rheumatoid arthritis or systemic lupus erythematosus), inflammatory bowel disease (for example, ulcerative colitis), irritable bowel disease (such as villous adenoma), gastrointestinal disorders caused by excess acids, pepsin or bile salts, Zollinger-Ellison syndrome, skin diseases or trauma (such as burns or acid or caustic injury), gout, Bartter's syndrome, fever, rheumatoid diseases, pain, and functio laesa.

Immunoregulatory diseases and diseases that can be treated using cranberry seed oil extracts of the invention and compositions derived therefrom include, but are not limited to, autoimmune diseases, for example, AIDS, chronic fatigue syndrome, graft rejections, and other viral diseases that impair the immune system.

Formulations and Methods of Administration
Cranberry seed oil extracts of the invention and compositions derived therefrom can be administered to a subject in any suitable form. For example, the extracts and compositions of the invention are sufficiently stable such that they can be readily prepared in a form suitable for adding to various foodstuffs including, for example, juice, fruit drinks, carbonated beverages, breakfast cereals, biscuits, cakes, muffins, cookies, toppings, bread, bagels, fiber bars, soups, crackers, baby formulae, salad dressings, cooking oils, and meat extenders.

In addition, cranberry seed oil extracts of the invention and compositions derived therefrom can be formulated as a pharmaceutical composition (e. g.. a medicinal drug) for the treatment of specific disorders.

In another embodiment, cranberry seed oil extracts of the invention and compositions derived therefrom can be formulated as a dietary supplement.

Suitable additives, carriers and methods for preparing such formulations are well known in the art.

For example, pharmaceutical compositions may take the form of tablets, capsules, emulsions, suspensions and powders for oral administration, sterile solutions or emulsions for parenteral administration, sterile solutions for intravenous administration and gels, lotions and cremes for topical application. The pharmaceutical compositions may be administered to humans and animals in a safe and pharmaceutically effective amount to elicit any of the desired results indicated for the compounds and mixtures described herein. In addition, the extracts of the invention may be used in cosmetics.

The pharmaceutical compositions of this invention typically comprise a pharmaceutically effective amount of a cranberry seed oil extract or fraction thereof containing, for example, a tocochromanol-containing cranberry seed oil extract, and if suitable a pharmaceutically acceptable carrier. Such carriers may be solid or liquid, such as, for example, cornstarch, lactose, sucrose, olive oil, or sesame oil. If a solid carrier is used, the dosage forms may be tablets, capsules or lozenges. Liquid dosage forms include soft gelatin capsules, syrup or liquid suspension.

Therapeutic and prophylactic methods of this invention comprise the step of treating patients or animals in a pharmaceutically acceptable manner with the compositions and mixtures described herein. As used herein, the term "pharmaceutically effective amount"refers to an amount effective to achieve a desired therapeutic effect, such as lowering blood levels of LDL-cholesterol and total serum cholesterol, while increasing the ratio of HDL-cholesterol to LDL-cholesterol, inhibiting lipogenesis, inhibiting platelet aggregation, decreasing the release of superoxides by human peripheral blood neutrophils. reducing levels of tumor necrosis factor or interleukin-1, reducing levels of arachadonic acid, increasing antibody titers in the blood, preventing thrombosis, preventing or treating inflammatory diseases, immunoregulatory diseases, fever, edema, diabetes mellitus, cancer, signs of aging, pain, septic shock, chronic fatigue syndrome and functio laesa; or decreasing the concentration of lipoproteins in the blood or to increase feed conversion efficiency.

The pharmaceutical compositions of this invention may be employed in a conventional manner for the treatment and prevention of any of the aforementioned diseases and conditions. Such methods of treatment and prophylaxis are wellrecognized in the art and may be chosen by those of ordinary skill in the art from the available methods and techniques. Generally, dosage ranges may be from about 1 to about 1000 mg/day. However, lower or higher dosages may be employed. The specific dosage and treatment regimens selected will depend upon factors such as the patient's or animal's health, and the severity and course of the patient's (or animal's) condition and the judgment of the treating physician.

The cranberry seed oil extracts of the invention and compositions derived therefrom also can be used in combination with conventional therapeutics used in the treatment or prophylaxis of any of the aforementioned diseases. Such combination therapies advantageously utilize lower dosages of those conventional therapeutics, thus avoiding possible toxicity incurred when those agents are used alone. For example, tocotrienols or tocotrienol-like compounds of the invention may be used in combination with bile acid sequestrants, such as Cholestyramine and Colestipol; fibric acid derivatives, such as, Clofibrate, Gamfibrozil, Bezafibrate, Fenofibrate, and Ciprofibrate; HMGR inhibitors, such as Lovastatin Mevastatin"", Pravastatin Simvastatin and SRI-62320; ProbucolTM; Nicotinic Acid (e. g., derivatives and conjugates). or estrogen antagonists, such as, for example, tamoxifen.

In foodstuffs, the cranberry seed oil extracts of the invention and compositions derived therefrom can be used with any suitable carrier or edible additive. For example, the cranberry seed oil extracts of the invention may be used as cooking oil, frying oil, or salad oil and may be used in any oil-based food, such as margarine, mayonnaise, or peanut butter. In addition, grain flour fortified with the compounds of the invention may be used in foodstuffs, such as baked goods (for example, breads, muffins. and pastries), cereals, pastas and soups. The cranberry seed oil extracts of the invention and compositions derived therefrom also can be emulsified and used in a variety of waterbased foodstuffs, such as drinks, for example, juice drinks, sports drinks, and drink mixes. Advantageously, the above-mentioned foodstuffs may be included in low fat, low cholesterol, or otherwise restricted dietary regimens.

Pharmaceutical compositions, dietary supplements, and foodstuffs of the present invention can be administered to humans and animals such as, for example, livestock and poultry. Once an animal has consumed or otherwise been administered the composition, it can advantageously retain the hypercholesterolemic, antithrombotic, antioxidizing, antiinflammatory, antiatherogenic, immunoregulatory, and other advantageous biological activities of the administered compounds. Accordingly, an animal raised under these conditions, or any product derived therefrom, such as, for example, milk, may be consumed by a human or another animal to derive the benefits of the cranberry seed oil extracts of the invention or compositions derived therefrom. For example, a chicken which ingests feed fortified with the extracts of the invention may later be eaten by a human to derive the cholesterol-reducing benefits.

In addition, the administration of the cranberry seed oil extracts of the invention or a composition derived therefrom can result in an increase in feed conversion efficiency. For example, in higher fat content animals, such as cattle, swine, sheep, and lamb, the tocotrienol containing cranberry seed oil extracts can advantageously lead to faster growth, lower cholesterol content, and higher percentage lean meat. When the compounds of the invention are administered to poultry, the tocotrienol containing cranberry seed oil extract can result in the production of eggs characterized by a reduced cholesterol content of the yolk and a higher protein content of the egg white.

Metliods for Extracting Cranberry Seed Oil
The novel extracts of the invention may be isolated from cranberry seeds using any suitable method, such the solvent system described in the examples provided below.

In a preferred embodiment, the invention provides an extraction method for isolating cranberry seed oil by physically disrupting the cranberry seeds, adding to the seeds an organic solvent to produce an extract/solvent mixture, and removing the solvent portion of the extract/solvent mixture such that an isolated cranberry seed oil substantially free of solvent results. In one embodiment of the extraction method, an isolated cranberry seed oil results that is suitable for use in a foodstuff, dietary supplement, or pharmaceutical composition. Other non-solvent based methods of extraction, such as cold pressing, can also be used.

Metitodsfor Isolating and Analyzing Specific Componentsfrom Cranberry Seed Oil
To isolate and analyze constituent components of cranberry seed oil, a variety of art-recognized techniques and assays can be employed. For example, as described in the studies provided herein, cranberry seed oil samples can be prepared for analysis by converting the fatty acids in the oil to their methyl esters, for example, by refluxing with MeOH/MeO-Na+. The resultant methyl esters can then be analyzed, e. g., by gas chromatography.

Sterol and triterpene alcohols can be extracted and analyzed using, for example, thin layer chromatography and high-performance liquid chromatography. For example, the isolated cranberry seed oil can be saponified with KOH, the unsaponifiables extracted with ether, and the resultant material can be fractionated on thin-layer chromatography (TLC) plates where the individual bands that are subsequently resolved can be scraped and extracted with a chloroform/methanol solvent. These resultant samples can then be analyzed using, e. g., gas and high-performance liquid chromatography (HPLC).

Phenolic compounds of cranberry seed oil can be analyzed and extracted using
HPLC analysis and solvent extraction, respectively. The isolated oil can be dissolved in hexane and then extracted with a methanol/water solution followed by centrifugation.

The extract can then be dried, and the residue can be resuspended in methanol/water for
HPLC analysis.

Tocochromanols contained in the cranberry seed oil of the invention can be separated and analyzed using, for example, the methods of Carpenter (Carpenter, Jr.,
A. P., J. Amer. Oil Chemists'Soc., 56: 668 (1979)).

Other methods known in the art may also be employed, in place of or in combination with, the methods described above for isolating cranberry seed oil components, particularly to"scale up"the quantity of the isolated components. For example, chromatographic techniques may be used for isolating either major or minor components of the cranberry seed oil of the invention, in sufficient and pure quantities, such that the component may be administered alone or as part of a composition or product described herein (e. g.,

foodstuffs, dietary supplements, pharmaceuticals, etc.).

In particular, gas liquid chromatography, gas solid chromatography, high pressure or high performance liquid chromatography (HPLC) (e. g., normal, reverse, or chiral), ion exchange chromatography, or size exclusion chromatography can be employed as described, for example, in Avances in Chromatography, Brown, Eds., Marcel Dekker,
Pub. (1998); Basic Gas Chromatography, Harold et al., John Wiley & Sons, Pub.

(1997); Column Handbookfor Size Exclusion Chromatography, Wu, Ed., Academic
Press, Pub. (1999); Fundamentals of Preparative and Nonlinear Chromatography,
Guichon et al., Eds., Academic Press, Pub. (1994); Handbook of Process Chromatography : A Guide to Optimization, Scale-Up and Validation, Hagel et al., Eds.,
Academic Press. Pub. (1997); HPLC Methodsfor Pharmaceutical Analysis, Lunn et al.,
John Wiley & Sons, Pub. (1997); and Practical High-Performance Liquid
Chromatography, Meyer, Wiley-Liss, Pub. (1999), each of which is incorporated by reference herein. Such isolated components, which can be separated as"value added" fractions (e. g.. fractions having therapeutic value), are typically rich in at least one selected major or minor component of the cranberry seed oil of the invention. These isolated components or fractions may be further combined to provide a composition rich in more than one component, including major components, minor components, and combinations thereof. In addition, a particular formulation intended for the treatment or prevention of a particular disease or condition may be formulated to be rich in those components having a therapeutic effect on the disease or condition (e. g., associated with affecting a change in any of the mechanisms associated with that particular disease or condition). For example, a formulation suitable for administering to a subject with cancer is preferably rich in cranberry seed components having antioxidant and other anti-cancer properties, whereas a formulation for administering to a subject with a dietary need, may be rich in, for example, beneficial fatty acids.

Metliodsfor Inhibiting Oxidation and Increasing Stability
In addition to the general precautions taken during the extraction process to avoid any unnecessary exposure to oxygen, e. g., protective blanketing of the extracts with carbon dioxide or nitrogen gas, the extracts and compositions derived therefrom of the invention may be further preserved by, for example, exposing the extracts to BHT, ascorbic acid, low temperature, or a combination of these conditions.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLE 1
METHOD OF EXTRACTING CRANBERRY SEED OIL (SMALL SCALE)
In this example, a method for performing small scale extractions of oil from cranberry seeds is described.

This extraction method is useful for extracting sufficient amounts of cranberry seed oil, for example, for performing laboratory analyses of its components.

Accordingly, the extraction method described herein allowed for the identification and characterization of each class of relevant components present in cranberry seed oil (see Example 3). The novel method is carried out as follows (see Fig. 1).

First, cranberry seeds are flaked without prior hull removal. This is in contrast to other oil seeds, which are usually dehulled first and then broken into grits that are in turn flaked. However. cranberry seeds are too small to conveniently submit to this procedure. The flaking step results in the disruption of each seed hull causing partial extrusion or expulsion of the seed meat which allows for efficient oil extraction.

Ideally, proper flaking produces flaked seeds having a speckled appearance due to the contrast between the yellow partly expelled seed meat and the outer red hull. It has been observed that improperly flaked seeds that are not speckled, i. e. are primarily red in appearance, do not extract as well as speckled seeds. Typically, the flaking step is carried out at room temperature rather than at higher temperatures (e. g., about 80 C) as is usually done with other oil seed grits. In addition, to protect the seed oil from unnecessary exposure to oxygen, the flaker is blanketed with an inert gas (nitrogen).

Next, cranberry seeds are mixed with dry ice prior to their being ground in a hammer mill in preparation for batch extraction. The use of dry ice creates a blanket of nitrogen which prevents overheating of milled material in choked sections of the hammer mill and greatly reduces the flow of oxygen-containing air through the mill and the ground seeds it contains. Cool, inert-gas blanketed flaking provides material that is extracted more readily, offer less flow resistance, and flows more uniformly through the mill and, thus, is superior to methods that involve grinding of the seeds.

After mechanical disruption of the cranberry seeds, the flakes were then exposed to the solvent hexane using the apparatus diagramed in Fig. 2. Typically, ground seeds were loaded into canisters to form a bed about 35 cm deep. A layer of corn starch was added as an inert material to assist in achieving an ideal flow rate of solvent through the seed material. Approximately six liters (4,152 grams) of hexane was used and allowed to percolate through the seed material at the flow rates indicated in tabular form below.

The final step of the method liberates isolated cranberry seed oil of a sufficient amount and quality for laboratorv analysis (see Example 3). The solvent may be removed using standard techniques. The oil concentrations from each run (as shown in
Table 6) were calculated from density measurements conducted using a pycnometer.

Table 6. Summary of Small Scale Extraction Data

Run Load in Solvent in Extract FlowTime Rate Liquid Conc. @
No. grams grams grams (hrs) cc/min Velocity 100 %
cm/min Yield
41523014E3350.310.3%11476
+160D
2 1449 1 2974 E 2878-4. 7 19 0. 16 21. 4 %
160 D
506H
3 1539 4152 H 2825 E 3. 5 28. 5 0. 24 6. 9%
+199D
2974E3233E2.7536.30.3115.1%41775
142D +96D
1262 H

EXAMPLE 2
METHOD OF EXTRACTING CRANBERRY SEED OIL (LARGE SCALE)
In this example. a method for performing a large scale extraction of oil from cranberry seeds is described.

This extraction method is useful for extracting sufficient amounts of high quality cranberry seed oil for commercial applications (e. g. as food additives, dietary supplements, pharmaceuticals, cosmetics, etc.). A flowchart depicting an overview of the large scale extraction process is provided in Fig. 3. The extraction method is described in detail under the following subsections, below.

Apparats
The large extraction apparatus (diagrammed in Fig. 3) consists of flaking and conditioning equipment, extractor, desolventizer, condenser, solvent and recovered solvent storage tanks, and a two-effect, steam-heated, tube-based. rising-film evaporator and associated condenser. In addition. the large scale apparatus also comprises a small, scraped-surface, vacuum evaporator (Luwa evaporator) with roughly 4 ft2 of heat-transfer surface and an associated condenser cooled by refrigerated water.

Flaking
An amount of 800 lbs. of dried cranberry seeds were processed in the pilot plant depicted in Fig. 4. Appropriate amounts of cranberry seeds were manually fed into the feed hopper of the Crown extractor. Periodically, proper flaking was monitored using under a small portable microscope. Acceptable flaking is characterized by seeds or partial polymerization would make recovery of trace ingredients from the oil much more difficult.

It is noted that much lower evaporation temperatures can be used if less stringent hexane removal is

employed. Only moderate hexane removal is required if the extracted oil is going to be subsequently processed to recover the minor constituents. The oil should be immediately cooled by passing it through a chilled heat exchanger as it leaves the evaporator. Since holdup times in scraped-surface evaporators are very short, a few seconds at most, the time of exposure of the oil to heat can be greatly reduced.

Cranberry seed flakes entered the extractor through a hopper near the right hand end of the top leg, were dragged into and through the system by the drag bars on the chain-link drive, descended through a vertical leg and entered the bottom leg where they moved from left to right, contacting progressively leaner and leaner extract. The flakes were then dragged upward through a vertical leg and were contacted by very lean extract and fresh solvent. Flakes were then passed over a drainage section where part of the interstitial extract in the bed discharged. The drained flakes were then dropped out of the extractor into an inclined drag conveyor which carried the flakes upward and deposited them in the top of the desolventizer, whose operation is described later. The vertical, descending bed of flakes at the left-hand side of the extractor prevents hexane vapor escaping from the extractor. Sight glasses were used in the extract application and drainage zones to monitor deposition and drainage of hexane from the flake bed. The particular extractor used in carrying out this method was seven feet long and 0.627-feet wide and typically allowed for a five-inch deep bed of flakes to be processed.

Under the above conditions, 157 lbs. of cranberry seed flakes per hour and 180
lbs. of extract per hour were fed into the extractor. The resultant extract/solid ratio ((E/R) = 1.15) was slightly higher than the ratio normally used (E/R = 0.8 to 1.0) for oil seeds in Crown extractors. Using these processing rates, a total of 625 lbs. of flakes were fed into the extractor and 425 pounds of desolventized flakes were discharged from the desolventizer. Fifty gallons of extract were produced. Based on a single sample whose density was 0.74 grams/cm3, and using a density versus concentration equation we developed (and correcting for small temperature effects) the extract concentration was determined to be about 24%. Accordingly, given the following calculation
50 gallons x 8.34 x 0.74 lbs./gallon = 308.6 lbs. of extract collected
308.6 lbs. x 0.24 (% concentration) = 74 lbs. of oil we determined that 74 lbs. of high quality cranberry seed oil were produced using the above method. This corresponds to a nominal yield of 11.8% (74 lbs. of oil/625 lbs. of flakes x 100% = 11.8%).

Extraction Yield Improvements
Abnormal operating conditions are used when starting up and shutting down a large extractor. Yield losses due to the need to accumulate material in the extractor during startup and due to material left in the extractor at shutdown cause nominal yields for short runs to be much lower than yields for steady-state operation. Accordingly, differences between the nominal yield for a short run and that for steady-state operation depend on the start-up and shut-down conditions used. Based on the occupied chain length, bed depth, width and bulk density, the extractor was estimated to contain 131
lbs. of flakes. In addition, solvent flow was not started until flakes reached the solvent inlet port (top, right-hand side) of the extractor. Thus. based on an estimate of the amount of extract held up in the system, it is more likely that extract discharge starts 55 minutes after solids feeding begins. This estimate suggests that no yield would be obtained during the 55 minutes of feeding, and the net amount of flakes fully subject to extraction during the run would only be 494 lbs. (625 lbs. of total flakes-131 lbs. of flakes held up in the system = 494 lbs. of flakes actively processed). Accordingly, using the method of the invention with the above considerations in mind, it is estimated that the yield obtainable from a steady-state operation would be as great as 74/494 x 100% = 15.0%. This corresponds to a yield of 15.0/21.5 x 100% = 69.7% based on an initial oil content of 21.5% for cranberry seeds.

Evaporation
The extract processed above was then evaporated to be substantially free of solvent under a vacuum and using no added heat. Alternatively, the extract can be processed as above by heating to 60 C (140 F) by using heating coils in the extract receiver (the receiver is normally maintained under a vacuum of 7-8 in. of H2O). When this is done, a great deal of solvent evaporates in the receiver, and the residual solvent can be readily evaporated in the Luwa evaporator (a scraped-surface, vacuum evaporator). Further, the extract can also be sent to a two-effect, tubular, rising-film evaporator (operating at slightly higher vacuum than the Luwa) where most of the solvent can be removed at operating temperatures close to that used in the Luwa. This partly desolventized extract can then be sent to the Luwa evaporator to remove even more hexane. Holdup times in the tubular evaporator are relatively long whereas those in the Luwa evaporator are quite short, only a few seconds.

A preferred evaporation method for gentler and shorter holdup times at high temperature employed the use of the Luwa evaporator without preheating the extract in the extract receiver. The Luwa evaporator was operated at a vacuum of 22 inches of Hg maintained using a steam jet ejector. In the evaporator, a rapidly rotating wiper acted on the extract as it flowed down the inner wall of a steam-heated tube. This provided very good heat transfer and minimized resistance of hexane transfer across the oil film.

Though high steam temperatures were used, fluid temperatures in the upper part of the evaporator were about 32 C, the boiling point of hexane in a vacuum of 22-in. of Hg.

As the extract fluid flowed down the tube, sufficient hexane evaporated to reduce its mole fraction in the extract to about 0.5, a weight fraction of about 9% and the fluid temperature was calculated as rising to about 50 C. If hexane still behaved fairly ideally when its concentration dropped to 4.5%, the fluid temperature would have risen to about 70 C.

Hexane exhibits large negative deviations from ideality at low concentrations.

Based on curves developed with cottonseed oil extraction, 220 F (105 C) of heat is needed to provide 2% residual hexane in cottonseed oil. Accordingly, steam pressure and the extract inflow rate were set to achieve a 220 F outlet temperature for oil leaving the evaporator. Hexane driven off from extract in the evaporator passed over into condenser cooled with refrigerated (10 C) water. Non-condensables and any hexane that did not condense, passed out of the system through the steam condenser used to maintain vacuum.

Product
A total of 36 lbs. of stripped oil (i. e., substantially free of solvent) were collected having a density of 0.92 gr/cm'at 80 F (27 C). This corresponds to roughly 0.926 grams/cm3 at 20 C. Based on a concentration versus density formula, this corresponds to 79% oil (based on a pure oil density of 0.995 gr/cm3). Most vegetable oils have densities in the 0.915 to 0.94 gram/cm3 range, and the listed density for, e. g., pure linseed oil, ranges from 0.92 to 0.94 gram/cm3.

Flake Recovery
The flakes were desolventized by successively passing them as a four or five-inch deep bed over three, steam-heated, circular trays in the desolventizer. The bed of flakes was slowly swept around the trays by rotating rakes and after a complete circuit on one tray fell through a choked opening in the tray onto the tray below. Trays were heated with 120 psig steam (350 F-177 C) and the solvent driven off in the desolventizer was condensed and recovered. A total of 425 lbs. of desolventized flakes were recovered with another 25 lbs. of flakes estimated to be hung up in niches in the equipment. Accordingly, it was determined that the 625 lbs. of seed flakes lost roughly (625-450) = 175 lbs. of weight or 175/625 x 100% = 28% of their original weight in the extraction process. This exceeds the estimated extraction yield of 15% for continuous extraction by 13%. The extra loss in weight is estimated to be largely due to evaporation or loss of moisture from the seeds during extraction and desolventization.

It is noted that the appearance of spent flakes discharged from the desolventizer changed during the course of the run. Initially, flakes were speckled and contained like amounts of white spots. Later they were redder and appeared to contain more intact seeds. This is an indication that flaking efficiency can decline during extraction and should be monitored in order to avoid suboptimal yields. Another variable which can lead to suboptimal yields is high bed permeability. A high bed permeability can reduce the amount of time flakes are in contact with solvent/extract. A simple equation can be used in carrying out the method of the invention. If A/R represents the amount of solvent absorbed or entrained per unit mass of flakes and if E/R/A/R-1 < 1, the fractional yield based on initial oil content can never be greater than E/R/A/R-1. In the present case, a relatively low E/R was used to obtain an oil-rich extract and reduce the amount of solvent that had to be removed in the Luwa extractor. Preferably, this aspect of the invention can be manipulated and a yield approaching 100% can be achieved by using a high enough E/R. To achieve such results, the A/R can be measured such that an appropriate E/R is set.

<#s> Conditioner
In carrying out the above described method of the invention, seeds fed to the flaking rolls were not exposed to heat. However, it is understood that a modification of the invention could also include heating the seeds (e. g., to a temperature of 170 F or 180 F) in a conditioner (a device containing swept, steam heated trays) prior to flaking.

This step can inactivate lipase and make the seeds easier to flake.

EXAMPLE 3
ANALYSIS OF THE COMPONENTS OF CRANBERRY SEED OIL
In this example, cranberry seed, isolated using the methods of the invention described above, was subjected to a detailed analysis of its major and minor components. Accordingly, a detailed description of the major and minor components of cranberry seed oil is described in the following subsections.

Major Components
Fatty Acid Composition
An analysis of the fatty acid composition of cranberry seed oil (CSO) was performed by converting an cranberry seed oil sample to its methyl esters by refluxing with MeOH/MeO-Na+ followed by refluxing with MeOH/HC 1. The methyl esters were then analyzed by gas chromatography using a Supelcowax 10 column (size, 30 m; i. d., 0.32 mm; film thickness, 0.25 mm). The carrier gas employed was Helium, the oven temperature and injection port temperature was 250 C, FID 260 C, and the program used was 1 min at 180 C, 180-220 C at 10 /min, 4 min at 220 C. The results of this analysis are provided below in Table 7.

Table 7. Fatty Acid Composition of Cranberry Seed Oil

Composition moles %
FattyAcid 1 2 3 4 Average
16:0 6. 7 6. 55 6. 4 6. 5 6. 5
18:0 1. 1 1. 1 1. 3 1. 2 1. 2
18 : 1 21. 1 21. 2 21. 2 21. 2 21. 2
18:2 38. 05 38. 0 38. 1 37. 8 38. 0
18:3 33. 05 33. 15 33. 1 33. 2 33. 1

The above results demonstrate that cranberry seed oil is unique in its high content of both linoleic acid (omega-6) and a-linolenic acid (omega-3) fatty acids.

In order to further characterize the fatty acid distribution in the isolated cranberry seed oil, the triglyceride composition in the oil was analyzed according to the triglyceride carbon number using gas chromatography. The column characteristics were as follows: DBl. L = 4.5 m ; film: 0.1 um; i. d. = 0.317; injection, on column : and gas;
Helium at 40 Kpa. The temperature program used was as follows:
180 C 1 mint 180 C 280 C 340 C 5 C/min. 340 C 2 min FID = 370 C.

Analyses according to ECN (ECN = Number of carbon atoms less 2 for each double bond present; glycerol carbon atoms not included in the count), is given Tables 8 and 9, below.

Table 8. Analysis of Fatty acids in Cranberry Seed Oil According to their ECN Numbers

NAME CONC RT AREA RF
ECN36 4. 658 8. 54 2443311 1. 000
ECN38 12. 330 10. 44 6467236 1. 000
ECN40 21. 837 12. 99 11453112 1. 000
ECN40 2. 734 15. 07 1434129 1. 000
ECN42 21. 124 17. 29 11079193 1. 000
ECN42 5. 793 19. 17 3038255 1. 000
ECN44 8. 185 22. 46 4293041 1. 000
ECN44 5. 646 23. 80 2961219 1. 000
ECN44 3. 489 24. 86 1830110 1. 000
ECN44 3. 516 26. 27 1843976 1. 000
ECN46 5. 124 31. 02 2687546 1. 000
ECN46 3. 073 34. 31 1611877 1. 000

ECN46 0. 263 36. 02 137941 1. 000
ECN48 1. 213 43. 46 636120 1. 000
ECN48 1. 015 47. 80 532182 1. 033
16 0. 000 65. 94 102807
TOTALS 100. 000 52552055

UNNORMALIZED TOTAL 5244924800.000
Table 9. Summary of ECN Analysis

NAME CONC
ECN36 4. 658
ECN38 12.330
ECN40 24.571
ECN42 26.916
ECN44 20.836
ECN46 8.460
ECN48 2.227
TOTAL 100.00
Stereo-Chemical Analysis
A further characterization of the fatty acids in cranberry seed oil was performed using stereo-chemical analysis. The analysis was conducted on a silica column using a purified sample in anhydrous ether and subsequently reacted with EtMgBr for 30 sec to obtain a limited decomposition (Table 10. Verification was done by thin-layer chromatography (TLC) using a hexane-ether solvent mix (50: 50, v/v), which permitted the separation of the sn-1,2 and the sn-23 diglycerides.

Calculation of the internal (Ai) and external (Ae) positions is conducted according to the following relationships.

Ai = 4 AD6a,-3At
Ae=3A6-Ai/2
where:
At=A in total glycerides
Ai = A in internal positions
ADGap-3At = A in the 1,2 and 2,3 positions
Ac = A in external positions
Results of the Stereo-Chemical Analysis are summarized in Table 10, below.

Table 10.

C16: 0 C18: 0 C18 : 1 C18: 2 C18: 3
% Mol. 6. 5 1. 2 21. 2 38. 0 33. 1
Aj (D) 5.0 (25 5) 1.3 (33. 4) 23.4 (36.7) 44.0 (38. 5) 26.3 (26.4)
Ae 7.2 (74.5) 1.0 (66.6) 20.1 (63.3) 35.1 (61.5) 36.5 (73.6)

It is clear from the above data that the fatty acids of cranberry seed oil. have a beta-position is rich in oleic (omega-9). linoleic (omega-6). and linolenic (omega-3) acids-a unique phenomenon in view of the stereo-selectivity of the human pancreatic lipase. Cranberry seed oil can thus be considered a potentially valuable source for application in medical research.

*Analysis of Minor Components Analysis of tlre Sterols and Triterpene Alcohols
In order to determine the particular sterol and alcohol content present in cranberry seed oil (see Table 11). the following protocol was used. An oil sample extracted according to the methods above was saponified using KOH and the unsaponifiable fraction was extracted with ether and fractionated on thin layer chromatographic plates coated with silica. The developing solvent consisted of anhydrous

hexane/ether/formic acid (S0: 50: 1. v/v/v). The bands corresponding to sterols. 4a-methyl sterols. and triterpene alcohols were scraped and extracted at ambient temperature with anhydrous HCC13/MeOH (90: 10, v/v). Qualitative and quantitative analysis of each class was carried out via gas chromatography, high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS). The amounts of the different classes were determined to be as listed below.

(a) Total Unsaponifiables in the oil 2.6%
(b) Total Triterpenic compounds:
in the unsaponifiables 51.4%
in the oil 1.3%
(c) Sterols in the unsaponifiables 30.7
in the oil 7982 mg. Kg~
in the triterpenic fraction 87.8% of which 60% were A5 and
27.8 % were A7 sterols
(d) Triterpene Alcohols
in the unsaponifiables 6.7% (or 1742 mg. Kg-1)
in the triterpenic fraction 12.2%
The presence of 4a-methylsterols was at very low concentrations and therefore not quantifiable by HPLC.

Further analytical detail was obtained for each class of components above.

Analysis of tlze Sterols
Cranberry seed oil was determined to contain several sterols (see Table 11) of which two were present in relatively large amounts (relative gas chromatographic retention times of 1. 33 and 1.45were noted). The mass spectra analysis of both sterols showed identical molecular ions at m/z 414 with the empirical formula C29Hsoo)
However, NMR spectroscopy indicated that one of the sterols had the intracyclic double bond at position 5 (i. e., A5) while the other sterol had a double bond at position 7 (i. e., A 7).

Accordingly, the major MS features of the A5 sterol were:
414 (M+, 82%), 396 (M-H2O, 93%), 381 (M-H2O-Me, 44%), 329 (M-H2O-CsH7* 13%) 303 (M-H20-CgHlo, 31%), 273 (M-CloH21,45%) 255 (273-H20,70%), 231 (273-C3H6-H, 49%), 213 (231-H20,100%)
And the major MS features of the A7 sterol were:
414 (M+, 75%), 399 (M-Me, 37%), 381 (M-Me-H2O, 14%), 273 (M-CloH21, 31%), 255 (273-H20,100%), 231 (273-C3H5-H, 34%), 213 (231-H20,58%)
Based on the above data, the A5 sterol was confirmed to be P-sitosterol (80.1 %), and the A7 sterol was determined to be schottenol (i. e., stigmastenol) (19.9%) as shown in Fig. 6.

Table 11. ANALYSIS OF THE STEROLS IN CRANBERRY SEED OIL

STEROL %
COLESTEROL 0. 08
24 METYLENCOLESTEROL 0.03
CAMPESTEROL 3.87
CAMPESTANOL 0.22
STIGMASTEROL 1.37
A7 CAMPESTEROL 1.19
(NK) 1.37
A5-23STIGMASTADIENOL 0. 66
CLEROSTEROL 0.43
PSITOSTEROL 59.97
(NK) 0.47
SITOSTANOL 0. 31
1.53#5AVENASTEROL
A5-24STIGMASTADIENOL 0. 47
(NK) 0.25
A7STIGMASTENOL 25.15
A7 AVENASTEROL 2.63

TOTALSTEROLS PPM 6574
PPM=PARTS PER MILLION
NK=NOT KNOWN (UNIDENTIFIED) Analysis of the Triterpene Alcohols
An analysis for the presence of triterpene alcohols in cranberry seed oil was performed and three major components were found (see Table 12). Two components were determined to be pentacyclic triterpene alcohols (having retention times of 1.36 and 1.50) and one component was determined to be tetracyclic triterpene alcohol (having a retention time of 1.65).

Mass spectral features of the first triterpene alcohol identified were: 426 (M+, 7%), 411 (M-Me, 17%), 393 (M-Me-H2O, 3%), 218 (M-H20-C 14H22, 100%), 203 (218-Me, 52%), 189 (218-C2H5, 16%). This compound was determined to be P -amyrin.

Mass spectral features of the second triterpene alcohol were: 426 (M+, 8%), 411 (M-Me, 8%), 393 (M-Me-H2O, 13%), 218 (M-H2O-C14H22,100%), 203 (218-Me, 21%), 189 (218-C2H5, 30%). This compound was determined to be a-amyrin.

Mass spectral features of the third triterpene alcohol were: 440 (M+, 16%), 422 (M-Me, 41%), 407 379(M-H2O-C3H7,55%),300(Me-C9H17,100%), 25%), 313 (M-C9H17-2H, 3%), 273 (M-C12-H22-H, 22%), 255 (273-H20,24%). This compound was determined to be 24-methylene parkeol.

The relative amounts of the triterpene alcohols were determined to be 9.9 % for p-amyrin, 44.8 % for a-amyrin, and 45. 3 % for 24-methylene parkeol. The chemical structure for each of the three major triterpene alcohols identified in cranberry seed oil is shown in Fig. 7.

Table 12. ANALYSIS OF THE ALCOHOLS IN CRANBERRY SEED OIL

TOTAL ALCOHOLS (ppm)20.53
%
C22% CH2 (CH2i) OH 32.29
C24% 26.06
C25% 1.95
C26% 13.88
C27% 3.17
C28% 22.65
TOTAL TRITERPENE ALCOHOLS (ppm) 1422.1
COMPOSITION OF TRITERPENE ALCOHOLS %
0.281NK
0.542NK
P AMYRIN 1.90
13.164NK
BUTYROSPERMOL 0.72
6, 646NK
1.037NK
1.788NK
CYCLOARTENOL 17.22
10 NK 0.23
11 NK 0.92
0.0612NK
24 METHYLENEPARKEOL 1.41
24 METYLENECYCLOARTENOL 13.55
0.1915NK
3.6716NK
5.3517NK
CITROSTADIENOL 29. 49
0.2419NK
20 NK 0.94

0.2521NK
22 NK 0.43

NK = Not known (unidentified) ppm=parts per million Analysis of the Phenolic Compounds
The phenolic compounds in the cranberry seed oil of the invention were determined using HPLC, MS, and UV spectral analysis. An initial analysis indicated that only small amounts of phenolic compounds were present. Accordingly, a larger sample of oil was used to improve detection and identification. Specifically, a 20 g sample dissolved in hexane (912.5 ml) was extracted with methanol/water (80/20 v/v) three times (12.5 ml each) and centrifuged. Next, the extract samples were dried in a rotary evaporator and the remaining residue was resuspended in 10 ml of methanol/water for separation by high performance liquid chromatography (HPLC) and identification by liquid chromatographic (LC)-electrospray negative mass spectrometry.

The following HPLC parameters were employed: SPHERISORB ODS-2 column, length 25 cm; i. d. 4 mm; detector DIODE ARRAY from 200 nm to 400 nm; linear gradient from 90 % A (water-0.5% H3P04), 10% B (acetic acid-methanol (50 50 v/v) to 50 % A B in 40', to 100 % B in 60'. The chromatogram was monitored at 280 nm. For quantitative analysis, 4-hydroxy-3-methoxycinnamic acid was used as internal standard.

Using the above methods of analysis, two major phenolic components were identified. The first component showed a specific absorption at 274 nm in UV analysis spectra (Fig. 8) had a shorter retention time than the second component (in reverse phase it is an index of more polarity), and, when subjected to electrospray negative mass spectrometry, gave a peak at m/z 180.69 (molecular ion), and with different cone voltage gave two important fragments at m/z 120.82 and at m/z 76.91 as shown in Fig.

9. Based on these the above findings, this compound was provisionally identified as methoxyphenylpropionic acid with the chemical structure indicated in Fig. 7.

The second predominant phenolic component identified in cranberry seed oil showed a specific absorption at 276 nm in UV analysis spectra (Fig. 8), and in electrospray negative mass spectrometry, had a molecular ion at m/z 178.71. In addition, this component displayed a principal fragment at m/z 118.72 as shown in Fig.

10. This compound was identified as methoxycinnamic acid with the chemical structure shown in Fig. 10.

Relative to the internal standard used. the concentrations of methoxyphenylpropionic acid and methoxycinnamic acid in cranberry seed oil were determined to be 1.8 ppm and 1.4 ppm, respectively.

Analysis of the Tocochromanols
Cranberry seed oil of the invention was analyzed for the presence of tocochromanols, a class of compounds that includes both tocopherols and tocotrienols.

The method used for quantitating these compounds is based on the ability of these compounds to reduce the ferric ions (Fe3+) to (Fe2+). In particular, tocochromanols in the presence of certain reagents (e. g., orthophenantroline) form an orange complex, the intensity of which can be measured by visible spectrometry conducted at 510 nm.

Further, absorption intensity is proportional to concentration, thus allowing for a determination of the amount of compound present in the sample.

Separation of individual tocopherols and tocotrienols was carried out using high performance liquid chromatography according to the method of Carpenter (Carpenter, Jr., A. P. J. Amer. Oil Chemists's Soc., 56: 668 (1979)). Detecting particular tocopherols or tocotrienols was conducted using UV absorption at 295 nm and identification was achieved by performing a comparison of retention times for the unknown components against known standards.

The qualitative and quantitative results of the analysis of the tocopherol and tocotrienol components of

cranberry seed oil

Table 13. Quantitative Analysis of Tocopherols and Tocotrienols

Rel. Retention time % mg. kg'
a-tocopherol (a) 1.00 6. 6 131
a-tocotrienol (aT3) 1. 07 9. 1 181
y-tocopherol (y) 142 5. 6 112
y-tocotrienol (y T3) 1. 58 75. 5 1505
6-tocopherol (6) 1.95 0. 8 16
8-tocotrienol (6T3) 21. 9 2. 4 48

Summary
The determinations described above reveal that the cranberry seed oil of the invention has a remarkably high amount of a-linolenic acid Only flaxseed oil contains a higher amount (-50%) of this omega-3 fatty acid; two oils. i. e., soybean and rapeseed, contain-7%; all other edible oils contain less than 2%.

In addition, and in contrast to flaxseed oil, cranberry seed oil also contains an equally high amount of the omega-6 fatty acid, linoleic (-38%). Further, stereochemical analysis of cranberry seed oil fatty acids indicated that the, p-position is rich in oleic (omega-9), linoleic (omega-6). and linolenic (omega-3) acids.

In addition, the above determinations show that cranberry seed oil is relatively rich in sterols, and triterpene alcohols, in particular, a-and p-amyrins. and 24methyleneparkeol.

EXAMPLE 4
IN VITRO ASSAY DEMONSTRATING THE ANTI-CANCER PROPERTIES OF
A CRANBERRY SEED OIL EXTRACT
The following studies were performed to examine the anti-cancer properties of cranberry seed oil extracts.

Two independently derived cranberry seed oil extracts (OS96 and OS97) were test for their ability to inhibit the growth of two different human breast cancer cell lines (i. e., MDA-MB-435 and MCF-7). In each case, the extracts of the invention demonstrated the ability to inhibit the growth of each tumor cell line with greater growth inhibition being seen against the estrogen receptor positive cell line MCF-7.

The in vitro assay was performed as follows. First, the human breast cancer cell lines MDA-MB435 (estrogen receptor-negative) and MCF-7 (estrogen receptor-positive) were cultured under standard conditions using, minimum essential medium (alpha modification, 3.7 gm of sodium bicarbonate per liter, 10% v/v fetal calf serum). Media for culturing MCF-7 cells was further supplemented with 1 mM sodium pyruvate, 10 ug/mL insulin, 1% v/v fungizone (antibiotic/antimycotic, 10,000 units/mL penicillin G sodium, 10,000 ug/mL streptomycin sulphate and 25 ug/mL amphotericin B in 0.85% saline)).

Next, cells were plated at a density of 2 x 104 cells/well in 96-well, flat-bottomed tissue culture plates in a total volume of 200 uL of medium and incubated at 37 C, with or without the cranberry seed oil extracts. The plates were incubated for 48 hours at 37 C and [3H] thymidine was then added to determine the number of dividing cells at each concentration of cranberry seed oil extract. The cells were reincubated for 4 hours, after which the medium and excess radiolabel were removed and cells were harvested and assayed for incorporated radioactivity as a measure of cell proliferation.

Accordingly, the percentage of dividing cells was determined by comparing the number of disintegrations per minute of the treated cells (average of 3 wells/concentration) with that obtained for the control cells. The concentrations at which 50 % and 90 % growth inhibition occurred was determined as the IC50 & IC90 for each extract. Results are presented in Table 14 and represent the average of 3 experiments + SEM.

In summary, both the OS96 and OS97 cranberry seed oil extracts of the invention exhibited potent growth inhibition of the tumor cell lines tested.

Table 14. The effect of cranberry seed oil extracts (OS96 and OS97) on the human breast cancer cell lines MDA-MB-435 (estrogen-receptor negative) and MCF-7 (estrogen-receptor positive)

MDA-MB-435

Extract IC50 (ug/mL) IC90 (ug/mL)
OS96 62. 5 + 4. 5 82. 9 + 5.3
OS97 15. 6 + 1. 1 29. 4 + 1.9
MCF-7
Extract IC50 (ug/mL) IC90 (ug/mL)
OS96 32. 6 + 2. 1 43. 7 + 2.6
OS97 7. 8+0. 4 12. 4+0.8 1

EXAMPLE 5
IN VITRO ASSAY DEMONSTRATING THE CHOLESTEROL LOWERING POTENTIAL OF CRANBERRY SEED OIL EXTRACT
The following studies were performed to examine the cholesterol lowering properties of cranberry seed oil extracts are demonstrated.

Two independently derived cranberry seed oil extracts (OS96 and OS97) were test for their cholesterol-lowering potential using a human liver cell line (HepG2). At least one of the tested extracts demonstrated the ability to reduce the amount of apoB secreted from the human liver cells. This was taken as a indication that the cranberry seed oil extracts of the invention are capable of causing beneficial changes in liver function relating to cholesterol metabolism.

The human liver cells (i. e., hepatoma HepG2 cells) of this example are known to secrete and catabolize lipoproteins similar to LDL and have been used as a model of human liver function relating to cholesterol metabolism. Thus, the ability the cranberry seed oil extracts of the invention to change HepG2 secretion of lipoproteins was assayed in order to determine if the extracts of the invention have cholesterol lowering potential.

The assay was performed as follows. First, Hep2G cells were cultured in minimum essential medium (supplemented with 10% fetal bovine serum or 1 % bovine serum prior to experimentation) and co-cultivated with a negative control extract (bovine serum albumin) or increasing concentrations (25-200 ug/mL) of cranberry seed oil extract made up in the same carrier liquid. After 24 hours of exposure to the extracts, the cell media was assayed for the presence of apolipoprotein B using an enzyme-linked immunosorbent assay (ELISA). In particular, cells were washed and dissolved in 0.1 N NaOH for protein determination and the apo B content of the medium was calculated in ug per mg of cell protein and expressed as percent of control (medium of cells incubated with DMSO) and these results are presented in Table 15.

The results show that increasing concentrations of the OS96 extract caused a dose-dependent reduction of apo B in the cell medium. The highest dose of OS96 (200 ug/mL) extract significantly lowered apo B in the medium by 34%. The apo B-lowering effect produced by the remaining doses was non-significant. In contrast, OS97 did not significantly affect levels of apo B in the medium at any concentration tested.

Thus, in at least one extract (OS96), a significant cholesterol lowering potential was observed as measured by a reduction in apo B levels in human liver cells.

Moreover, using a MTT assay to assess cell viability, it was determined that none of the cranberry seed oil extract dosages tested were toxic to cells.

Table 15. Changes in overall apo B production in HepG2 cells exposed to increasing concentrations of cranberry seed oil extracts

Extract N Conc, ug/mL Percent apo B in medium
OS96 4 100 + 6
OS96 4 200 66 + 9
OS96 4 100 71 + 17

OS96 4 50 83+7
OS96 4 25 92+ 7
OS97 4 0 100 + 13
OS97 4 200 80+16
OS97 4 100 93 + 10
'OS97"4'5084+7
OS97 4 25 100 + 20

Means + SEM. * = significant different from control, p < 0.05 EQUIVALENTS
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

<#s> What is claimed

Claims

1. An isolated cranberry seed oil extract substantially free of impurities.

2. The extract of claim 1 comprising a tocochromanol selected from the group consisting of a-tocopherol, y-tocopherol, b-tocopherol. a-tocotrienol, y-tocotrienol, and 6-tocotrienol.

3. The extract of claim 1, further comprising an exogenous flavonoid, tamoxifen, or a combination thereof.

4. The extract of claim 3, wherein said flavonoid is a flavone, flavavone, isoflavone, or flavonol.

5. The extract of claim 1, further comprising a fatty acid.

6. The extract of claim 5, wherein said fatty acid is a-linolenic acid (omega-3), oleic acid (omega-9), linoleic acid (omega-6), or a combination thereof.

7. The extract of claim 1, further comprising a sterol.

8. The extract of claim 7, wherein said sterol is p-sitosterol, schottenol, or a combination thereof.

9. The extract of claim 1, further comprising a triterpene alcohol.

10. The extract of claim 9, wherein said triterpene alcohol is a-amyrin, p- amyrin, 24-methyleneparkeol, or a combination thereof.

11. The extract of claim 1, further comprising a phenolic compound.

12. The extract of claim 11, wherein said phenolic compound is methoxyphenylpropionic acid, methoxycinnamic acid. or a combination thereof.

13. A foodstuff comprising an extract according to claim 1. or one or more components derived therefrom.

14. A dietary supplement comprising an extract according to claim 1, or one or more components derived therefrom.

15. A pharmaceutical composition comprising an extract according to claim 1, or one or more components derived therefrom.

16. A method for treating or preventing a disease or condition in a subject comprising the step of administering to said subject a therapeutically-effective amount of a foodstuff, dietary supplement or pharmaceutical composition of claims 13,14 or 15, respectively.

17. The method of claim 16, wherein said disease or condition is selected from the group consisting of a malignancy, a hypercholesterolemic-related disease, a thrombotic disease, a respiratory disease, an atherogenic disease, an inflammatory disease or condition, a neurological disease, a dermatological disease, an opthalmological disease, or a gastroenterological disease.

18. The method of claim 16, wherein said subject has, or is at risk for acquiring, a malignancy and wherein said composition comprises a tocotrienol, a flavonoid, and tamoxifen.

19. The method of claim 16, wherein said subject has or is at risk for acquiring a hypercholesterolemic-related disease and wherein said composition comprises atocopherol, a-tocotrienol, y-tocotrienol, 6-tocotrienol, or a combination thereof.

20. The method of claim 16. wherein said subject has, or is at risk for acquiring, a respiratory disease, an inflammatory disease or condition. a neurological disease, a dermatological disease, an opthalmological disease, or a gastroenterological disease and wherein said composition comprises a-tocopherol.

21. A method for treating, preventing, or lowering the risk of acquiring a disorder or condition associated with an alteration in membrane stability, membrane fluidity, 5-lipoxygenase activity, or protein kinase C activity in a subject comprising administering to said subject a therapeutically-effective amount of a

foodstuff, dietary supplement or pharmaceutical composition of claims 13,14 or 15, respectively.

22. The method of claim 21, wherein said administering is orally.

23. A method of nutritionally supplementing a foodstuff comprising, adding to said foodstuff an extract according to claim 1, or one or more components derived therefrom.

24. A method for isolating cranberry seed oil comprising,
-physically disrupting cranberry seeds; -adding to seeds an organic solvent to produce an extract/solvent mixture; and
-removing the solvent portion of the extract/solvent mixture
thereby producing isolated cranberry seed oil substantially free of solvent.

25. The method of claim 24, wherein said method further comprises the step of separating the extract/solvent mixture from the cranberry seeds.

26. The method of claim 24, wherein said isolated cranberry seed oil is at least 70% free of solvent.

27. The method of claim 24, wherein said isolated cranberry seed oil is at least 80% free of solvent.

28. The method of claim 24, wherein said isolated cranberry seed oil is at least 90% free of solvent.

29. The method of claim 24. wherein the organic solvent is hexane.

30. The method of claim 24, wherein said adding is conducted at a temperature between 50 and 90 F.

31. The method of claim 24, wherein said adding is conducted at a temperature between 50 and 65 F.

32. The method of claim 25, wherein said separating is performed in an extract receiver maintained at room temperature and atmospheric pressure.

33. The method of claim 24, wherein said removing is conducted at a temperature between 30 and 220 F and under vacuum.

34. The method of claim 33, wherein said vacuum pressure is 22 inches of Hg or greater.

35. The method of claim 24, wherein said producing results in a yield by weight of at least 10% or greater.

36. The method of claim 35, wherein said yield by weight is at least 15% or greater.

37. The method of claim 35, wherein said yield by weight is at least 20% or greater.

38. The method of claim 24, further comprising the step of increasing the oxidative stability of the extract.

39. The method of claim 38, wherein said increasing is performed by exposing the extract to ascorbic acid, BHT, low temperature. or a combination thereof.

40. Isolated cranberry seed oil produced by the method of claim 24.

41. The isolated oil of claim 40 substantially free of impurities.

42. The isolated oil of claim 41, comprising tocochromanol selected from the group consisting of a a-tocopherol, y-tocopherol, 6-tocopherol, a-tocotrienol,, y- tocotrienol, 6-tocotrienol, or a combination thereof.

43. The isolated oil of claim 40, further comprising an exogenous flavonoid, tamoxifen, or a combination thereof.

44. The isolated oil of claim 40, comprising a compound selected from the group consisting of a-linolenic acid (omega-3), oleic acid (omega-9), linoleic acid (omega-6), p-sitosterol, schottenol, a-amyrin,-amyrin, 24-methyleneparkeol, methoxyphenylpropionic acid, methoxycinnamic acid, or a combination thereof.

45. The isolated oil of claim 41, in a form suitable for use in a foodstuff.

46. The isolated oil of claim 41, in a form suitable for use as a dietary supplement.

47. The isolated oil of claim 41, in a form suitable for use in a pharmaceutical composition.

48. A foodstuff comprising the oil of claim 41.

49. A dietary supplement comprising the oil of claim 41.

50. A pharmaceutical composition comprising the oil of claim 41.
